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Cooked ham: effect of spent *Saccharomyces* yeast extract addition in the technological process and evaluation of global quality

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Author's Declaration

Under the terms of the "Decreto-lei nº 216/92, de 13 de Outubro" is hereby declared the following original articles were prepared in the scope of this thesis.

Under the terms of the referred "Decreto-lei", the author declares that he afforded a major contribution to the conceptual design and technical execution of the work, interpretation of the results and manuscript preparation of the published articles included in the thesis.

Publications in International Peer-Reviewed Journals

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A. Alves, O. Viegas, **G. Pacrazio,** A. Lemos, O. Pinho. IMPLVO Ferreira. Addition of spent yeast extract on the production of cooked ham: compositional and sensory evaluation. 7th Meeting of Young Researches of University of Porto, Book of Abstracts 12th to 14th February 2014, pp. 231.

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Abstract

Cooked ham is a widely consumed meat product. France, Spain and Italy are the major consumer countries in Europe. This meat product is a typical luncheon product, with pink colour and characteristic flavour. Its usual process of production includes several stages, such as tumbling and/or massaging, cooking and cooling. The addition of non-meat proteins is a common practice to reduce production costs and to improve yield in meat industry. However, the addition of spent yeast extract from brewer industry was not study yet.

Brewery spent yeast biomass is the second by-product from brewing industry. The spent yeast extract used in this work was obtained from ready-to-discard yeast biomass that was appropriately extracted. The cell wall was removed and the extract was lyophilized to be used as a new ingredient in cooked ham production.

In the experimental work of this thesis, conventional cooked hams produced by the industry with 18 h of tumbling, cooked 1.5 h and weighing around 900 g were characterized at the beginning and at the end of shelf life concerning its physical and chemical properties. Afterwards, cooked ham production was modified to reduce its production time. Thus, tumbling time was reduced in 50% and different cooking times, namely, 1.5 h, 2 h, 2.5 h and 3 h were tested. Part of these cooked hams was prepared with addition of 1% spent yeast extract. Another part of these cooked hams that were used as control suffered similar processing but were not added of spent yeast extract, only the acetate buffer used in the preparation of yeast extract was added. The physical and chemical properties of these cooked hams were also evaluated at the beginning and at the end of shelf life and the results were compared with the characteristics of conventional cooked ham. Sensorial analyses were carried out at the ham industry by a trained panel to evaluate the impact of reducing tumbling time and add spent yeast extract on organoleptic characteristics of the final product at the beginning and at the end of shelf life.

Comparison between conventional cooked (18 h of tumbling / 1.5 h of cooking) and cooked ham with 9 h of tumbling and the same cooking time shown that the effect of tumbling time was significant on physical characteristics, namely colour and texture, whereas the chemical characteristics were less affected since proteolysis of myofibrillar

proteins, free amino acids (FAA) content and volatile profile was similar. The effect of shelf life was most relevant on chemical characteristics, increased the proteolysis of myofibrillar proteins and the relative % of some volatiles, mainly, aldehydes, whereas FAA decreased, except histidine that increased.

Concerning the effects of spent yeast extract addition as an ingredient in production of cooked hams with reduced tumbling time it was observed that spent yeast extract addition increased hardness, gumminess, chewiness, ash and protein contents. Some FAA, namely, Ala, Gly, Val, Ser, Thr, Lys, Trp also increased, whereas qualitative volatile profile was similar. The influence of cooking time was less prominent. No advantages were observed on increasing cooking from 1.5 h to 3 h. During shelf life cooked hams with spent yeast extract presented similar evolution as control that did not contain spent yeast extract. The higher hardness of cooked ham added with yeast extract is due to the strength of the gel formed during cooking that improves the textural characteristics, thus this additive is a good gel stabilizer in cooked ham formulations.

In general, sensorial characteristics of cooked hams added of spent yeast extract (9 h tumbling time), without addition of spent yeast extract (9 h tumbling time) and conventional ham (18 h tumbling time) all cooked during 1.5 h were not significantly different. At 12 days no significant differences were observed between these three types of ham concerning texture and flavour. Nevertheless, significant differences were observed between cooked ham with and without spent yeast extract with regard to colour and between cooked ham with spent yeast extract and conventional cooked ham in global appreciation, being the first one the lower scored by panellists. At the end of shelf life the sensorial characteristics of cooked ham added with spent yeast extract were similar to those of cooked ham without spent yeast extract and with those of conventional ham for all attributes and presented a global appreciation of good. Accordingly, the physical and chemical modifications due to the effect of tumbling time, yeast extract addition and shelf life had no significant impact on sensorial characteristics of cooked ham. Spent yeast extract can be used as a new ingredient in the production of cooked ham to reduce the tumbling time of conventional cooked ham without compromising its quality characteristics.

Resumen

El jamón cocido es un producto cárnico consumido mundialmente. Francia, España e Italia son los países con mayor consumo en Europa. Este producto cárnico es un típico producto de refecciones, de flavour y color rosado característico. Su usual proceso de producción incluye distintas etapas como, masaje por caída y/o masajeado por rotación, cocinado y enfriamiento. La adición de proteínas no cárnicas es una práctica común utilizada para reducir costos y mejorar la ganancia en la industria cárnica. Mientras tanto, la adición de extracto de levadura reutilizado proveniente de la industria cervecera no fue estudiado aún.

La biomasa de levadura descartada es el segundo subproducto de la industria cervecera. El extracto de levadura utilizado en este trabajo fue obtenido y apropiadamente extraído de la biomasa de levadura lista para descartar. La pared celular fue removida y el extracto fue liofilizado para ser usado como un nuevo ingrediente en la producción de jamón cocido.

En el trabajo experimental de esta tesis, los jamones cocidos fueron producidos de manera convencional por la industria con 18 h de masaje por caída, cocinadas durante 1.5 h y pesando alrededor de 900 g, fueron caracterizados al inicio y al final de su vida útil teniendo en cuenta sus propiedades físicas y químicas. Más tarde, la producción de jamón cocido fue modificada para reducir su tiempo de producción. En consecuencia, el tiempo de masaje por caída fue reducido en un 50% y diferentes tiempos de cocción, principalmente, 1.5 h, 2 h, 2.5 h y 3 h fueron evaluados. Una parte de estos jamones cocidos fueron preparados con la adición de 1% de extracto de levadura usada. La otra parte de estos jamones cocidos que fuera usada como control sufrió un procesamiento similar pero no se le adicionó extracto de levadura usada, solo fue adicionado el buffer acetato usado en la preparación del extracto de levadura. Las propiedades químicas y físicas de estos jamones cocidos fueron también evaluadas al inicio y final de su vida útil y los resultados fueron comparados con las características de los jamones cocidos convencionales. Fue realizado un análisis sensorial en la industria cárnica por un panel entrenado para evaluar el impacto de la reducción en el tiempo de masaje por caída y adición de extracto de levadura usada sobre las características organolépticas del producto final al inicio y final de su vida útil.

La comparación entre jamón cocido convencional (18 h de masaje por caída / 1.5 h de cocción) y jamón cocido con 9 h de masaje por caída y el mismo tiempo de cocción muestran que el efecto de tiempo del masaje por caída fue significativa sobre las características físicas, principalmente color y textura, mientras que las características químicas fueron afectadas en menor medida desde la proteólisis de las proteínas miofibrilares, el contenido de amino ácidos libres (FAA) y el perfil de los compuestos volátiles fueron encontradas similares. El efecto de la vida útil fue más relevante en las características químicas, incrementando la proteólisis de las proteínas miofibrilares y el área relativa % de algunos volátiles, principalmente, aldehídos, mientras que los FAA decrecieron, excepto histidina que aumentó.

En relación a los efectos de la adición de extracto de levadura usada como ingrediente en la producción de jamón cocido con tiempo de masaje con caída reducido se observó que la adición de extracto de levadura usada aumentó la dureza, gomosidad, masticabilidad, contenido de cenizas y proteínas totales. Algunos FAA, principalmente, Ala, Gly, Val, Ser, Thr, Lys, Trp también aumentaron su concentración, mientras que el perfil de compuestos volátiles cualitativamente fue similar. No se observaron ventajas en el incremento del tiempo de cocción de 1.5 h a 3 h. Durante el período de vida útil los jamones cocidos con adición de extracto de levadura usada presentaron evoluciones similares a los controles que no poseían adición de este extracto. El aumento en la dureza de los jamones cocidos adicionados con extracto de levadura usada es debido a la fuerza del gel formado durante la etapa de cocción que mejora las características texturales, por lo que este aditivo es un buen estabilizador de gel en formulaciones de jamón cocido.

En general, las características sensoriales del jamón cocido adicionado con extracto de levadura usada (9 h de masaje con caída), sin adición de extracto de levadura usada (9 h de masaje con caída) y jamón cocido convencional (18 h de masaje con caída) todos con un tiempo de cocción de 1.5 h no fueron significativamente diferentes. A los 12 días, no se observaron diferencias significativas entre estos tres tipos de jamones en relación a textura y flavour. Sin embargo, diferencias significativas fueron observadas entre jamones cocidos con y sin la adición de extracto de levadura usada con respecto a color y entre jamón cocido con extracto de levadura usada y jamón cocido convencional en apreciación global, siendo el primero el menos puntuado por los panelistas. Al final de la vida útil las características sensoriales del jamón cocido adicionado con extracto de

levadura usada fueron similares a aquellas de jamón cocido sin extracto de levadura usada y a aquellos producidos convencionalmente para todos los atributos y presentando una apreciación global de “Buena”. En consecuencia, las modificaciones físicas y químicas debido al efecto del tiempo de masaje por caída, adición de extracto de levadura y vida útil no tuvieron un impacto significativo en las características sensoriales del jamón cocido. El extracto de levadura usada puede ser utilizada como un nuevo ingrediente en la producción de jamón cocido para reducir el tiempo de masaje por caída de jamón cocido convencional sin comprometer su característica sensorial.

Resumo

O fiambre é um produto à base de carne mundialmente consumido. França, Espanha e Itália são os principais países consumidores da Europa. O fiambre é um produto tipicamente utilizado em merendas, apresentando um *flavour* e uma cor rosada caraterísticos. O processo habitual de produção inclui várias etapas tais como *tumbling* e/ou massagem, cozinhar e arrefecimento. A adição de proteínas não cárneas é uma prática comum para reduzir os custos de produção e aumentar o rendimento na indústria da carne. No entanto, a adição de extrato de levedura da indústria cervejeira ainda não foi estudada.

A biomassa da levedura cervejeira é o segundo subproduto mais abundante da indústria cervejeira. O extrato de levedura utilizado neste trabalho foi extraído a partir da biomassa de excedente de levedura. Após remoção da parede celular o extrato foi liofilizado para ser usado como um ingrediente na produção de fiambre.

No trabalho experimental desta tese, fiambres convencionais produzidos pela indústria, pesando cerca de 900 g, com 18 h de *tumbling* e cozidos 1,5 horas, foram caracterizados no início e no final do tempo de prateleira, relativamente às suas propriedades físicas e químicas. Entretanto, a produção do fiambre foi modificada com o objetivo de reduzir o seu tempo de produção. Assim, o tempo de *tumbling* foi reduzido em 50 % e foram testados diferentes tempos de cozedura, nomeadamente, 1,5 h, 2 h, 2,5 h e 3 h. Uma parte destes fiambres foi preparada com adição de 1 % de extrato de levedura. Outra parte, usada como controlo, teve um processamento similar mas sem adição de extrato de levedura, incluiu apenas a solução tampão de acetato utilizado na preparação do extrato de levedura. As propriedades físicas e químicas destes fiambres foram avaliadas quer no início quer no fim do tempo de prateleira e os resultados foram comparados com as características do fiambre convencional. Análises sensoriais foram realizadas na indústria do fiambre através de um painel treinado para avaliar o impacto quer da redução do tempo de *tumbling*, quer da adição do extrato de levedura, nas características organolépticas do produto final, no início e no fim do tempo de prateleira.

Na comparação entre o fiambre convencional (18 h de tumbling / 1,5 h de cozedura) e o fiambre com 9 h de *tumbling* e o mesmo tempo de cozedura, verificou-se que o efeito do tempo de *tumbling* foi significativo sobre as características físicas, isto é, na cor e na

textura, enquanto as características químicas foram menos afetadas, isto é, a proteólise de proteínas miofibrilares, o conteúdo de aminoácidos livres (FAA) e o perfil aromático foram semelhantes.

Tendo em conta o efeito da adição do extrato de levedura como um ingrediente na produção de fiambres com tempo de *tumbling* reduzido, foi observado um aumento na dureza, gomosidade, mastigabilidade, cinzas e conteúdo em proteína. Alguns FAA, como Ala, Gly, Vai, Ser, Thr, Lys, Trp, também aumentaram, enquanto o perfil qualitativo de voláteis foi semelhante. A influência do tempo de cozedura foi menos proeminente. Não se observaram vantagens no aumento do tempo de cozedura de 1,5 h para 3 h. Durante o tempo de prateleira, os fiambres com extrato de levedura apresentaram evolução semelhante aos controlos que não continham extrato de levedura. A maior dureza do fiambre com adição de extrato de levedura pode dever-se à força do gel formado durante a cozedura, que melhora as características de textura, nesse sentido este aditivo é um bom agente estabilizador do gel nas formulações de fiambre.

No geral, as caraterísticas sensoriais do fiambre com adição do extrato de levedura (9 h de *tumbling*), do fiambre sem adição do extrato de levedura (9 h de *tumbling*), e do fiambre convencional (18 h de *tumbling*), todos cozinhados durante 1,5 h, não foram significativamente diferentes. Aos 12 dias não foram observadas diferenças significativas entre os três tipos de fiambre relativamente à textura e ao *flavour*. Não obstante, foram observadas diferenças significativas entre o fiambre com e sem a adição do extrato de levedura em relação à cor, e entre o fiambre com a adição do extrato e o fiambre convencional na apreciação global, tendo o primeiro menor pontuação pelo painel. No fim do tempo de prateleira, as características sensoriais do fiambre adicionado com extrato de levedura foram semelhantes às do fiambre sem a adição do extrato e às do fiambre convencional, para todos os atributos e apresentaram uma apreciação global "Boa". Deste modo concluiu-se que as modificações físicas e químicas devidas à redução do tempo de *tumbling*, adição de extrato de levedura e tempo de prateleira não mostraram um impacto significativo nas características sensoriais do fiambre. O extrato de levedura pode ser usado como um novo ingrediente na produção de fiambre para diminuir o tempo de *tumbling* no fiambre convencional sem comprometer a sua qualidade.

Scopes and aims

Cooked ham is a very common brine-treated product consumed worldwide. In Europe, France, Spain and Italy are the major consumers. Different manufacturing technologies are used for its production; additionally variations in raw material can occur. Cooked ham manufacturers invest much effort from an economical point of view to obtain a final product with good sensory and quality characteristics.

Researchers worldwide are looking for new ingredients extracted or produced from industry by-products because reutilization and valorisation of these materials is becoming an increasingly important investigation topic for the next years.

Brewer's *Saccharomyces* yeast biomass is the second major by-product from beer industry. Because the increase of beer consumption worldwide, solutions for valorisation of *Saccharomyces* yeast biomass are welcome.

Dried yeast biomass can be used in food industry due to its content in proteins, nucleotides, minerals and vitamins. Yeast products are usually found in the form of powders, flakes or tablets, or in liquid form. However, the biomass from brewing industry is generally sold for animal feed after inactivation by heat.

For this reason the present work was centred in the reutilization of brewing *Saccharomyces* yeast biomass (spent yeast extract) from a local brewer industry (UNICER) to produce an ingredient to be applied as ingredient in the production of cooked ham. Hence, it was essential to determine the sensory and quality characteristics of conventional product and compare with cooked ham added of spent yeast extract aiming to reduce tumbling/massaging and cooking steps. Understand the effect of spent yeast extract in quality and sensory characteristics of cooked ham at the beginning and at the end of shelf life is relevant to prevent that consumers reject this new formulation.

The overall aim of this thesis is:

To produce a cooked ham with addition of spent yeast extract and reduced production time in comparison with the conventional product, maintaining similar quality and sensory characteristics.

To achieve this goal the first step was the characterization of conventional cooked ham at the beginning at the end of shelf life. Afterwards, the impact of 50% reduction of tumbling time, and addition of spent yeast extracts on physical, chemical and sensorial characteristics of cooked ham at the beginning and at the end of shelf life was studied. Finally, comparison was performed with the characteristics of conventional cooked ham.

The results obtained in the experimental work were organized in three sections according to steps mentioned previously

Section A: Conventional cooked ham: Characterization of its quality attributes at 12 and 90 days

- Determination of the proximate composition.
- Evaluation of textural and colour attributes and comparison with bibliography.
- Understand the proteolysis that occurs by evaluation of protein hydrolysis and free amino acids (FAA) content.
- Analyses of volatile compounds profile.
- Evaluation of the modifications that occur during shelf-life by comparison of cooked ham composition at 12 and 90 days of production (shelf-life period).

Section B. Cooked ham with reduced tumbling time: effects of spent *Saccharomyces* yeast extract addition and cooking time.

- Evaluation of the proteolytic activity of spent yeast extract and the influence of incubation temperature (4°C and 70°C).
- Determination of the proximate compositions of cooked ham added of acetate buffer (control) and spent yeast extract cooked during different times.

- Understand the contribution of spent yeast extract and cooking time on the texture, colour, proteolysis (evaluation of protein hydrolysis and FAA) and volatile compounds of cooked ham ready to consumption (12 days).
- Evaluation of the modifications that occur during shelf-life of cooked ham with and without addition of yeast extracts by comparison of composition at 12 and 90 days of production (shelf-life period).

Section C. Sensorial characteristics of cooked ham with and without spent S*accharomyces* yeast extract: relation with its physical and chemical parameters

- Evaluation of sensorial characteristics of cooked ham with and without yeast extract
- Understand de quality attributes related with mouth texture, colour, flavour and global appreciation of cooked ham

Abbreviations

AA	Amino acids
ANOVA	Analysis of Variance
BSTFA	bis-trimethyl silyl trifluoro acetamide
CAR/PDMS	Carboxen/Polydimethylsiloxane
CIE	Commission Internationale d'Eclairage
CW	Carbowax
DFD	Dark, firm and dry
DVB	Divinylbencene
EPA	Environmental protection agency
FAA	Free amino acids
GC	Gas chromatography
GC-MS	Gas chromatography-Mass spectrometry
GRAS	Generally Recognized as Safe
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HS	Headspace
IS	Internal standard
LC	Liquid chromatography
LLE	Liquid/liquid extraction
LOD	Limits of detection
LOQ	Limits of quantification
MTBSTFA	N-Methyl-N-(tertbutyldimethylsilyl) trifluoroacetamid
NIST	National Institute o Standards and Technology
NP	Norma Portuguesa
OPA	o-phtaldialdehyde
PA	Polyacrilate
pA	Peak Area
PAGE	Polyacrilamide Gel Electropheresis
PCA	Principal component analysis
PDMS	Polydimethylsiloxane
PSE	Pale, soft and exudative
PSDB	Proteins soluble in diluted phosphate buffer
PSSIB	Proteins soluble in strong ionic buffer
RA	Relative areas

RP	Reversed-phase
R_T	Retention time
SAFE	Solvent assisted flavour evaporation
SDE	Solid phase extraction
SDS	Sodium Dodecyl Sulfonate
SIM	Single Ion Monitoring
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SPSS	Statistical Product and Service Solutions
TIC	Total ion current
TFA	Trifluoroacetic acid
TPA	Texture Profile Analisys
UV	Ultraviolet
WBS	Warner Bratzerl Shear

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Chapter 1.

Introduction

1 Cooked ham

Cooked ham is a very common brine-treated product consumed worldwide, for retail and catering, for export and domestic markets. In Europe its consumption represents about 26% by volume of the delicatessen products commercialised (Casiraghi, Alamprese et al. 2007). The biggest consumers are Spain, France and Italy (Utrera, Armenteros et al. 2012). Manufacturers aim to produce cooked ham that is microbiologically safe and of high organoleptic quality (Tomović, Jokanović et al. 2013). Thus, they are continually looking for new technologies to deliver better ham products to the consumer while maintaining minimum production costs.

According to Portuguese NP 4393-2001 (Portuguees Institue of Quality 2002), ham classification is determined by pieces of meat used in the production thereof and can be categorized in: *fiambre da perna superior, fiambre da perna extra, fiambre da perna, fiambre da pá and fiambre corrente*.

1.1. Raw material

The selection of raw material is of major relevance to guarantee the sensory quality of cooked ham (Toldrá, Mora et al. (2010). However, the wide breed variety and feeding may frustrate the standardization of products. Thus, the advances in process technology and their versatility aid to maintain the quality of cooked ham (Xargayó 2010a). Consequently, it is usually mentioned that raw material is as important as technology process to obtain cooked ham of high quality (Casiraghi, Alamprese et al. 2007).

Cooked ham consists mainly in meat cuts from the hind leg of the pig (Codex Alimentarius 1981), and parameters like water holding capacity, pH and fat of meat should be controlled. Water holding capacity is linked to ham pH (Toldrá, Mora et al. 2010), values between 5.8 to 6.2 may assure a good water retention. Two types of ham are referred in literature pale, soft and exudative (PSE) and dark, firm and dry (DFD) hams. These characteristics are also linked with pH, PSE presents low pH (<5.6) and low water holding capacity, consequently higher cooking loses occur and the final product presents lower colour (Toldrá, Mora et al. 2010, Brauer 2009). On the other hand, DFD ham facilitates

water retention and dark colour, but due to the higher pH (>6.3) it is more susceptible to microbial growth (Xargayó 2010a).

Fat content is another important parameter of raw material, because it influences flavour development. The fat content and composition depends on the feed given to pigs (Toldrá, Mora et al. 2010). Fat has a technological interest to solve problems that can result from binding different muscles and the cooking loss. Nevertheless, the fat that is between muscles tends to retain juice giving a spongy appearance. The amount of tolerable fat by the consumer depends on the social customs of each region or country. In Europe, the presence of fat contributes not only to the traditional aspect, but also to the characteristic taste of each type of ham, since fat contributes significantly in the development of taste, whereas in the United States it is required that the product is sold completely free of fat (Xargayó 2010a).

The second major ingredient is water; it is used in almost all cooked hams depending on the type and quality intended. Water must be of high microbiological and chemical quality. Ion content strongly affects cooked hams quality, if water contains ions like Ca^2+, Mg^2+ and heavy metals, the ions will affect negatively water holding capacity, and iron salts can destroy ascorbic acid activity and in some cases present toxicological risks (Freixanet 2010).

Salt (NaCl) used in cooked ham production is around 2% (Toldrá, Mora et al. 2010, Li, Szczepaniak et al. 2011) and plays an important role in ham production. Salt reduces water activity, solubilizes meat proteins, expanding its quaternary structures. Salt represents the main contribution to the ionic strength of the product, thus, weakening the electrostatic bonds between the groups COO^- and NH_4^+. Consequently, salt contributes to the water retention and the connection between the muscles in the finished ham (Freixanet 2010).

Other ingredients can be used in cooked ham production, some of them are optional, depending on the quality of the final product. Examples of these ingredients are sucrose, invert sugar, dextrose (glucose), lactose, maltose, glucose syrup (including corn syrup), honey, spices, seasonings and condiments, aromatics, hydrolysed protein and food grade gelatine (Codex Alimentarius 1981). Most of these ingredients are described as components of brine.

Sugars play an important role in cooked ham, not only to reduce water activity but also to intensify the product sapidity (Toldrá, Mora et al. 2010). Depending on the final quality of cooked ham, different sugars can be used, namely sucrose, dextrose, lactose, fructose, glucose syrup or/and dextrin's (Freixanet 2010).

Hams may also contain addition of proteins and hydrolysates to increase the protein content of the final product and water retention (Toldrá, Mora et al. 2010). Proteins of different origins can be used namely those that derivate from milk, eggs, blood, collagen and vegetables.

Starches can be used, in countries where their use is permitted by law, to increase water retention of cooked ham. Starch gelling by heat and make a tri-dimensional gel that retains high quantities of water. The best performance in cooked ham production is obtained with corn starch and cassava starch (Freixanet 2010).

A variety of flavouring agents are used in meat industry to produce cooked ham, including wine, fruit juices, vegetables protein hydrolysates, natural spices, spice teas, vegetables, smoke extracts, among others.

Production of cooked ham also includes a wide variety of additives, such us, colorants, nitrites and nitrates, preservatives, antioxidants, phosphates, flavour enhancers, stabilizers and thickeners.

The most used colorant is cochineal carmine because of its pink colour similar to cooked meat colour. This colorant is stable at light, pH and heat treatment (Freixanet 2010). Other colorants used are Annatto extract and hemoglobin but they have de negative aspects that are affected by pH, light or heat treatment. The hemoglobin must be used in high quantities to guarantee an optimal colour due to its light instability.

Nitrite and nitrate salts of sodium and potassium (E249, E250, E251, E252) are allowed as preservatives that can be added to cured meat products. Nitrite is a potent inhibitor of microorganisms, (Ferreira and Silva 2008) that protect against *Clostridium botulinum* and other *Clostridium* species (Council 2006). Additionally, contributes to the ham red colour and antioxidant activity (Toldrá, Mora et al. 2010). The principal action of nitrites is done by nitrous oxide. The free nitrous oxide react partially with myoglobin given nitroso myoglobin that it is the responsible pigment of colour in cooked ham. The other part of

nitrous oxide not fixed by myoglobin has different destinies that oblige to add levels of 125 to 250 ppm of nitrites to products to guarantee product quality.
Nitrate is used as a source of nitrite contributing to colour stability and preservative action after cooking, nitrates levels of 75 to 150 ppm are used. Thus, at industry it is a common routine to use a mixture of both nitrite and nitrate (Freixanet 2010).

Plant extracts and essential oils are widely used as preservatives because they contain active components that act as anti-microbiological and antioxidant agents. Adequate concentrations must be applied because in low concentrations they are not perceived by consumers, whereas at high quantities can give sensory characteristics not appreciated by consumers.

In some countries, salts of sorbic acid, benzoic acid and lactic can be added as preservatives, since these components have de capacity to reduce water activity of products.

The antioxidant most widely used around the world is sodium L-ascorbate. This compound has three activities in cooked ham production. First of all acts as reducing agent against nitrite, reducing it to nitrous oxide performing nitroso hemoglobin and accelerating cooked ham colour. If sodium ascorbate is used in cooked ham production, final quantities of nitrites in final products will be lower. Secondly, ascorbate acts helping to colour stability of final product inhibiting free peroxides radicals' formation in ham surface due to the combined action of ultraviolet light and oxygen. At last, it prevents the formation of carcinogenic nitrosamines blocking the formation of nitrosating agents (N_2O_3) from nitrous oxide.

Phosphates increase the pH values of the ham increasing water retention, far from the isoelectric point of meat proteins, but also increase the ionic strength and contribute to protein solubilisation (Toldrá, Mora et al. 2010). Depending on characteristics of final products, different polyphosphates are used, tripolyphosphate, pyrophosphate and hexametaphosphate. Both the first and the last are hydrolysed in aqueous medium gradually releasing pyrophosphate which is the responsible for the action of phosphates on the ham. Addition of 5 g/kg of phosphate is enough to produce good quality products.

Stabilizers and thickeners are also useful in ham production, namely carragenates that are polysaccharides derived from red algae. The main action of carragenates is stabilizers of

gel affecting and maintaining the hardness, flexibility, transparency, colour and syneresis. Addition of 1 to 5 g/kg is appropriate to guarantee water retention.

Concerning flavour enhancers, the most widely used is monosodium glutamate, 0.2 to 1 g/kg can be used in cooked ham production. Other flavour enhancers are used, such as sodium iosinate and sodium guanilate.

Yeast extracts produced from the yeast cells have been used as a flavouring agents in soup, sauces, gravies, stews, snack food, canned food and meat products (York and Ingram 1996, Halász and Lásztity 1991, Chae, Joo et al. 2001, Nick 2006). Interestingly yeast extracts have received much attention recently as flavour enhancers in low-sodium cured meat products (Desmond 2006). Yeast extracts offer alternatives for formulation of brines resulting in enhanced value added meat products (Walsh, Martins et al. 2010).

1.2. Processing technology

According to Tomović, Jokanović et al. (2013) the cooked ham quality could be influenced by many factors, which include, as mentioned before, the selection of raw material, brine composition and injection, mechanical treatment, and cooking/cooling treatment. The technological processing of cooked hams summarised in flow diagram of Figure 1 has significant effects on colour, water-holding capacity, cooking loss and texture, and consequently a substantial influence on consumer acceptance (Moretti, Bellagamba et al. 2009).

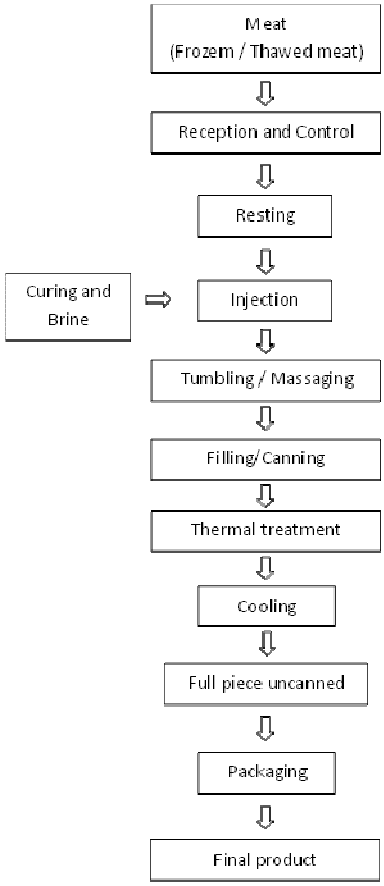

Figure 1: Typical flow of production of cooked hams adapted from Toldrá, Mora et al. (2010)

Traditionally, the cooked ham process consists in meat reception, brine injection, tumbling and/or massaging, cooking and cooling. All steps will be described in more detail but with emphasis in brine injection, tumbling and /or massaging, cooking and cooling.

Cooked ham process starts with the reception of pork meat pieces. They are received refrigerated and or frozen/thawed, pH and weight will be measured to control PSE or DFD ham. Bones are usually removed (Toldrá, Mora et al. 2010).

1.2.1. Brine injection, tumbling and /or massaging

The processing of cooked ham involves de addition of additives and ingredients that are required to improve colour and flavour. These additives are added to the brine and applied by injection into meat pieces (Xargayó 2010a).

The main brine ingredient is salt; all other additives mentioned earlier can be added. According with Toldrá, Mora et al. (2010) brine is injected into meat using a multineedle injector system. Brine is essential to promote the binding of muscles and protein solubilisation, increase flavour, give a better yield, a higher final weight and uniform distribution of sodium chloride, nitrites and other possible ingredients (i.e. sugars, spices, polyphosphates, etc.). The brine injection levels are characteristics of each product and determine the cooked ham quality (Casiraghi, Alamprese et al. 2007).

Massage and/or tumbling is important to distribute the brine and to increase its distribution into muscle. This process facilitates the extraction of proteins that cause binding of meat pieces and colour development (Krause, Plimpton et al. 1978). Cellular disruption, extraction and solubilisation of myofibrillar proteins in the presence of salt and phosphate have beneficial effects on increasing cooking yield, (Siegel, Theno et al. 1978, Hullberg and Lundström 2004, Szerman, Gonzalez et al. 2007, Cassidy, Ockerman et al. 1978, Xargayó, Lagares et al. 2010). Theno, Siegel et al. (1978) found that muscle fibres were disrupted after tumbling for 2 h with brine containing 2% salt and 0.5% phosphate, and myofibrils were detached with increasing protein solubility after 4 h tumbling (Whiting 1988) and tumbling time is important to obtain an acceptable product (Li, Szczepaniak et al. 2011)

1.2.2. Cooking

Cooking requires a rigorous control of time and temperature to achieve a final product with desirable quality (Toldrá, Mora et al. 2010). Cooking can be considered as pasteurization, since internal temperature of the ham reaches up 72ºC for 30 to 60 minutes (Toldrá, Mora et al. 2010). During cooking physicochemical, biochemical and microbiological phenomena's occur and will define quality and organoleptic properties of the finished

product (Lagares 2010). Thus, the main objectives of this step are development of sensory properties, microbial destruction and enzyme inactivation (Lagares 2010, Toldrá, Mora et al. 2010). Cooking denatures the myofibrillar proteins that are extracted and solubilized as a result of brine and massage effects, binding the muscles and making the ham slice compact (Casiraghi, Alamprese et al. 2007).

Two types of heat transfer mechanisms occur, namely convention (heat transfer from the heating medium to the ham surface) and conduction (heat transfer mechanisms from the ham surface to inner areas) (Toldrá, Mora et al. 2010).

The most used cooking system in ham industry is immersion in water (Zell, Lyng et al. 2012). This process has excellent capacity to transmit heat leading to easy temperature control, good homogeneity and short cooking times. However, it has some drawbacks, such as risk of microbiological contamination and high boiler dimensions. Steam oven is another option for cooked hams production; it is a discontinuous process with longer cooking times and a less heat exchange and transmission. This system is preferable for products with high cooking lose, cooked without vacuum (Lagares 2010).

Other alternatives for cooking process have been considered to replace immersion in water, but none of these cooking methods, such as infrared or microwave are used by the meat industry due to the lack of temperature uniformity during the cooking process (Lagares 2010).

Three different temperature diagrams can be applied in the cooking process: *i)* constant cooking temperature from beginning to the end of the thermal processing, *ii)* decreasing temperature and *iii)* increasing temperature. The first option is the most extensively used and presents overall acceptable results. Decreasing temperature is the traditional method of cooking ham. It begins with a high initial external temperature (80-90ºC) that is maintained until the centre of the piece reaches a predetermined temperature (50 – 55ºC), followed by a decrease of external temperature (70-75ºC) until finish the cooking process. In comparison with the previous method it gives lower yield, shorter shelf-life, as well as negative effects of the surface of final products and lack of cohesion in the slices (Lagares 2010).

Cooking ham with increasing temperature (*iii*) can be performed by two different ways: a) cooking at staggered temperature, temperature increase in a graded fashion, in successive

stages, until reach the desired temperature; b) Delta T cooking (ΔT), external temperature is increased continuously. These processes avoid excessive heating of the surface of the ham (Toldrá, Mora et al. 2010) and generate better organoleptic characteristics than previously described methods, but cooking times are much higher. Consequently, they are not viable and rarely used at industry (Brauer 2009, Lagares 2010).

According to Toldrá, Mora et al. (2010) the cooking efficiency, weight loss, and yields may be different for cooking methods at a fixed internal temperature or cooking by steps. It has been reported by Desmond and Kenny (2005) that slow heating rate forms a protein network with better water binding and less jelly losses resulting in low cooking damage, better slice cohesion and a more tender product.

1.2.3. Cooling

Cooling has a strong influence on the quality of the ham. It assures that the thermal centre of the ham achieves temperatures below 4-5ºC and it can be done by air blast, immersion in cold water, or cold water showers. Pre-cooling through shower or immersion in water allows a rapid reduction of internal temperature. The cooling from 40 to 15ºC is considered a critical period and should be restricted to less than 4 hours when possible (Toldrá, Mora et al. 2010). Slow cooling conditions may be dangerous, due to the exposure during long periods at relatively high temperatures that allow microorganisms growth. Vacuum cooling was reported to offer reduced cooling rates when compared with air blast, water immersion, or cold room, but it affects toughening, yield and quality (Toldrá, Mora et al. 2010).

1.3. Changes in protein configuration during ham processing

Brine injected into muscles causes protein changes due the strong ionic charge of brine components, namely, salt and phosphate, whose purpose is solubilizing myofibrillar proteins (Xargayó 2010b). According to Brauer (2009) the phosphate addition, especially diphosphates, has a very positive side effect on the tenderness of the cooked ham separating actin and myosin and braking down the muscle proteins. Additionally,

phosphates decrease meat pH. Other substances, such as, citrate, acetate, lactate and tartrate, can cause muscle proteins swell, but do not have the same results as phosphate concerning water-binding capacity.

Tertiary and quaternary structures of polypeptidic chains result from electrostatic bonds, hydrogen bridges, disulfide bridges, and bridges made up of divalent cations, specially calcium and magnesium. The protein´s water holding capacity during the cooking process will increase proportionally as tertiary and quaternary structure become less compact. This expansion is obtained by breaking the greatest number possible of these bonds. Increasing the medium´s ionic strength through the action of salt will reduce the electrostatic bonds. Phosphates can exert a chelating action on calcium and magnesium, loosening the bonds formed by these metals and allowing protein expansion (Freixanet (2010).

Meat massaging causes damage in muscle structure causing an increase in brine sorption, consequently, myofibrils and other muscle proteins are extracted to intercellular spaces and to the outside (Lachowicz, Sobczak et al. 2003). The main constituents of the exudates in massage, myosin and actin proteins are going to play a principal function in binding all pieces of meats used in cooked ham production (Siegel, Theno et al. 1978). However, the extracted proteins are unable to contribute to the binding, and for that reason the increase of ionic strength is needed to solubilize these proteins and improve the binding quality after thermal process of the final product (Siegel, Theno et al. 1978).

During tumbling, more rigorous than massage, the meat falls from the upper part of a rotating drum or strikes with paddles or baffles involving energy impact on the muscle causing alterations in muscle tissues (Cassidy, Ockerman et al. 1978, Krause, Plimpton et al. 1978). Tumbling has many beneficial effects in the ended product, such as, formation of protein exudates which will act as a sealer between muscles when proteins are denature during thermal processing, improve tenderness and colour homogenization (Cassidy, Ockerman et al. 1978, Li, Szczepaniak et al. 2011).

After protein solubilisation due to the effects described previously, the thermal process will denature muscle proteins properly by heat effect, which will cause a reduction in the intercellular spaces carrying out the formation of a tridimensional network able to hold water and give the typical characteristics to the final product (Lagares 2010). Products

without the addition of phosphates, usually contain collagen that ensures a good level of cohesion in the final product (Lagares 2010).

1.3.1. Flavour and aroma formation

Flavour and aroma of cooked ham is influenced by biochemical changes and reactions that occur during the production, mainly due to enzymatic reactions such as lipolysis and proteolysis. Muscle proteases and lipases contribute to the formation of free amino acids and fatty acids, which have strong effects on taste and aroma (Toldrá, Mora et al. 2010).

Proteolysis is enhanced by the water activity and salt content of cooked ham, but enzymes are easily inactivated above 50°C during cooking because its stability decrease quickly. However, there is a release of amino acids by muscle enzymes, aminopeptidase, which contribute to the flavour. The release depends on the length of time and heating intensity during cooking stage (Toldrá, Mora et al. 2010).

Lipolysis is also favoured by cooking ham conditions, especially when the pH is near neutral. Fatty acids are released before cooking stage, serving as substrates for generation of volatile compounds. The cooked ham flavour will depend on the time and intensity of heating (Toldrá, Mora et al. 2010). Lipid oxidation generates volatile compound, such as unsaturated aldehydes that are not present in fresh cooked ham (Toldrá, Mora et al. 2010).

The contribution of microorganisms to the flavour of cooked ham is irrelevant, but if present in ham they are responsible for acidification and formation of off - flavours during storage (Samelis, Kakouri et al. 1998, Toldrá and Flores 2006). The refrigerated storage of sliced cooked ham under vacuum or modified atmospheres leads to changes in the sensorial characteristics of the product, namely, formation of off - odours. Therefore, the refrigerated storage of sliced cooked ham modifies the volatile composition due to the metabolism of lactic acid bacteria that generates typical fermentation products, such as methyl branched alcohols and aldehydes (Leroy, Vasilopoulos et al. 2009)

1.3.2. Colour stabilization

The colour of cooked ham arises from a very complex development involving a large number of inter-connected reactions that take place during ham processing. Nitrite is added

to the cooked ham together with ascorbate/isoascorbate. The nitrite become nitrogen oxide that reacts with the myoglobin from the meat resulting nitrosomyoglobin, that gives the cured reddening (Brauer 2009). Heat causes denaturalization of nitrosomyoglobin and stabilization of is compound is obtained at the final cooking stage with temperatures near 65ºC. For this reason the optimal processing temperatures are between 65-75ºC to guarantee a good development and colour stabilization (Lagares 2010) .

The intensity of the pink colour depends on the initial content of myoglobin, which is associated with the type of muscle and the age of the animal, being higher in older animals (Aristoy and Toldrá 1998). It was reported that red colour is negatively correlated with sensory analysis by consumers. Increase of redness was considered less acceptable, this means consumers prefer cooked hams with light colour and less red colour (Válková, Saláková et al. 2007).

1.3.3. Microbiological stabilization

Thermal processing is ruled by the parameters of temperature and exposure time that will lead to the microbial destruction. If the initial microbial load is very high, severe heat treatments are needed. Moreover the product´s sensorial characteristics will also be affected.

To achieve an optimum degree of microbial safety of cooked cured meat products it will be necessary that the centre of the product maintain a constant temperature of 68°C or 70°C for 30 to 60 minutes. Another relevant aspect is the speed at which the temperature increases during cooking, since slow speeds can give rise to bacterial stress and development of thermal-resistant strains. Therefore, it is important to limit or reduce the time that the product remains exposed to temperatures favourable to thermal tolerance (40-50°C) (Lagares 2010).

1.3.4. Changes of ham composition during shelf-life

The flavour of cooked ham is thermally developed during cooking, mainly from amino acids and lipids (del Pulgar, Garcia et al. 2013). Strecker degradation of amino acids and

Maillard reactions produce a considerable number of branched and heterocyclic volatile compounds with low odour thresholds, also responsible for the flavour of ham. Lipid oxidation is another source of volatile compounds in cooked ham, specially the oxidation of unsaturated fatty acids (del Pulgar, Garcia et al. 2013). During ham storage the formation of volatile compounds will continue.

Ham heat treatment is much less than that required for commercial sterility. Thus, ham is a highly perishable meat product, since it is subjected to modifications in its structure, composition and properties during storage before consumption. Physico-chemical and microbiological changes are "translated into" sensorial deterioration. Consequently, ham shelf-life is around 90 days, defined as the time period during which it remains effective, useful, and suitable for consumption.

The traditional approach used for the establishment of ham shelf-life, consists of setting a cut-off point along the storage period when any of the measured attributes exceeds a pre-established limit. Shelf-life ends before food loses its original quality and/or safety attributes.

2 Enzyme extracts for meat industry

Enzymes can be added as ingredients to increase meat quality. These can be from different sources, such as plant or microbiological origin. According to (Sullivan and Calkins 2010), the United States federal agencies only accepts five exogenous enzymes – papain, ficin, bromelain, *Aspergillusoryzae protease*, and *Bacillus subtilis* protease – that are known as Generally Recognized as Safe (GRAS).

Several research works describe the use of plant and microbiological enzymes to improve meat quality in different types of products. Table 1 summarizes the application of plants enzymes in meat products. Ficin, bromelain and papain are the most widely used enzymes for meat tenderization (Dransfield and Etherington 1981) along with microbial proteases derived from Aspergillus species, these enzymes have regulatory approval (U.S.D.A) for improve meat quality and have been used in various forms as marinades, injection in brine, pre-slaughter injection into the animal´s vascular system, and incorporation into various spices as meat tenderizers (Ashie, Sorensen et al. 2002).

Table 1: Plants enzymes and plant extracts used to improved quality characteristics in meat products

Enzymes	Muscle	Animal	Effects	Author
Papain	Beef Top	Bovine	Papain has very limited specificity on meat proteins and acts only on myofibrillar proteins, but not connective tissue; its activity is self-limiting, thereby eliminating the risk of over tenderization.	Ashie, Sorensen et al. (2002)
Cucumis Trigonus Roxb; Ginger Rhizome	*Biceps femoris muscles*	Buffalo	Tenderizing effect; a significant reduction in shear force values	Naveena, Mendiratta et al. (2004)
Ginger powder	Breast and leg muscle	Bangalore and Coorg Spent-Hen varieties	Very effective in tenderizing the tough meats and in improving the sensory-flavor quality	Bhaskar, Sachindra et al. (2006)
Ginger Rihizome Extract	*Biceps femoris muscles*	Chevon	Decrease in shear force value	Pawar, Mule et al. (2007)
Actinidin	*Biceps femoris muscles*	Pig	Actinidin reduced WB shear force and improved sensory-assessed tenderness of cooked pork biceps femoris	Christensen, Tørngren et al. (2009)
kiwifruit juice	*longissimus dorsi (LD) muscle* and hind leg cut	Lamb	Produce degradation of myofibril proteins and the simultaneous appearance of new peptides	Han, Morton et al. (2009)
kiwifruit juice *(Actinidia Chinesis)*	*Bovine Semitendinosus*	Bovine	Could reduce the increase hardness in beef bulgogi induce by over 400 MPa	Lee, Oh et al. (2009)
Cucumis Trigonus Roxb; Papain; Ginger Rhizome	Muscles of hind legs	Sheep	Good tenderizing effect and higher acceptability than control	Mendiratta, Sharma et al. (2010)
Ginger Rihizome Extract	*Longissimus dorsi muscles*	Yak	Decrease in shear force value	Ruitong, Zhi et al. (2010)
Papain; Bromelain; Ficin; Ginger	*Triceps Brachii; Supraspinatus*	----	Improvements in both sensory and instrumental measures of tenderness.	Sullivan and Calkins (2010)
kiwifruit juice	*Longissimus muscle*	Pork	Resolved toughness associated with the temperature abuse.	Liu, Xiong et al. (2011)
Papain, Bromelain, Actinidin, Zingibai, Kiwi crede juice	*Semimembranosus muscles*	Bovine	Affected the volatile profile of cooked beef.	Ma, Hamid et al. (2012)
Calotropis procera latex	---	Pork, beef and chicken	Characteristics of the treated meat samples were improved	Rawdkuen, Jaimakreu et al. (2013)
Soy sauce	*biceps femoris*	Bovine	Collagen solubility and myofibrillar fragmentation index, contributing to decreased shear force.	Kim, Choi et al. (2013)

Ficin, the sulfhydryl protease of the fig tree (*Ficus carica*) had been used as a beef tenderizer by many authors who reported that it hydrolyzes and increases solubility of muscles proteins (El-Gharbawi and Whitaker 1963, Matulis, Wu et al. 1999, Ramezani, Aminlari et al. 2003). Aminlari, Shekarforoush et al. (2009) gives a brief description of the most popular beef tenderizer, Bromelain, that is composed of a number of protease enzymes and is extracted from the stems of pineapple plants to be concentrated.

Papain, extracted from the papaya, is another popular beef tenderizer (Lewis and Luh 1988). Also, papain is very heat-stable and, therefore, not readily inactivated, allowing continued product texture deterioration even after cooking (Ashie, Sorensen et al. 2002). As a disadvantage, as Miller, Strange et al. (1989) describe, papain can produce in a meat product and over tenderization due its very broad specificities to the major muscle proteins (connective tissue/collagen and myofibrillar proteins).

Another plant enzyme is cucumin. This is a promising enzyme from a melon variety *Cucumis trigonus Roxb*, has been reported to have proteolytic activity (Hujjatullah and Baloch 1970). The cucumis plant is found in India, Afghanistan and Persia. *Cucumis trigonus Roxb* (locally known as kachri) are traditionally local meat tenderizer in India (Naveena, Mendiratta et al. 2004).

‘‘Zingibain’’ or ginger rhizome, according with Naveena, Mendiratta et al. (2004) was first isolated from *Zingiber officinale roscoe* in 1973. The ginger protease is a thiol proteinase with an optimum activity at 60 ºC and the same author had demonstrated that Zingibian has proteolytic activity against collagen and actomyosin. However, ginger rhizome is used primarily as a flavouring agent for bakery products and is a widely used spice in a variety of food products in general and meat-based foods in particular. But, Ginger extract not only has proteolytics properties (Lee, Sehnert et al. 1986), but it also has antioxidant, antimicrobial properties as Bhaskar, Sachindra et al. (2006) that help extend the shelf life of a product. However, the use of fresh ginger imparts a pungent odour to the meat and may mask the meat´s flavour, also the same author refer that this extract has low proteolytic activity at low temperatures (4ºC) and the tenderizing effect can be increased at approximately 28ºC (Bhaskar, Sachindra et al. 2006).

Actinidin is another proteolytic enzyme from plant origin, it is a sulfhydryl protease found in the fruit of Chinese gooseberry (*Actinidia chinansis*) or kiwi fruit. Little information is

available on the application of actinidin as beef tenderizer and in manufacturing beef products (Aminlari, Shekarforoush et al. 2009)

Like plant enzymes, microbiological enzymes can be applied to meat products to improve their organoleptic properties, resistance to cutting and consumer acceptance. Mei, Liaw et al. (1998) said that microbial proteases are another source of potent meat tenderizers; and Takagi, Kondou et al. (1992) have demonstrated that Alkaline Elastase from an *Alkalophilic Bacillus* Strain properties are advantageous for specifically cleaving elastin and collagen, which are the main components of connective tissues in meat. This enzyme therefore seems to be more effective than papain or bromelain. Attempts have been made with success to use microbial that ensure controlled breakdown of meat proteins collagenases (Allen Foegeding and Larick 1986, Miller, Strange et al. 1989). However, these collagenases have not attracted much interest, due to potential pathogenicity of the enzyme source (purified collagenase produced by *Clostridium histolyticum* (Allen Foegeding and Larick 1986) and collagenase derived from Vibrio B-30 (Miller, Strange et al. 1989).

Yeasts from different sources are rich in proteolytic enzymes, however, their use requires extraction with appropriate procedures from the inner of yeast. Probably this is the major reason why yeast enzymatic extracts have hot extensive application. Currently, yeast extracts have received much attention as flavour enhancers in low-sodium fermented sausages, in low-sodium cured meat products and such as sauces, gravies, soups, chips and crackers (Desmond 2006, Campagnol, dos Santos et al. 2011).

Brewer's yeast is generally recognized as safe (GRAS) and has good nutritional characteristics (Chae, Joo et al. 2001). It is rich in numerous enzymes, namely, vacuolar proteases including serine, aspartyl, and metallo proteases, among others (Ferreira, Pinho et al. 2010). Thus, Brewer's *Saccharomyces* biomass is an ideal candidate for enzymes production. It is the second major by-product from brewing industry; its use is still limited, being basically used as animal feed (Ferreira, Pinho et al. 2010). *Saccharomyces sensus tricto* includes *S. Bayanus, S. cariocanus, S. cerevisiae, S. kudriavzevii, S. mikatae and S. paradoxus* (Wunderlich and Back 2009) and are by far the most used in the brewing industry. However, no studies are described concerning its proteolytic activity effects on meat tenderization, or as an ingredient in the production of cooked ham, thus the industrial application of these enzymes is a field to explore.

3 Analytical methodologies for evaluation of cooked ham quality

Quality of cooked ham should be addressed in a comprehensive way, from the viewpoint of chemical, physical and sensorial properties (Válková, Saláková et al. 2007). According to Delahunty, McCord et al. (1997) appearance, colour, flavour and texture are main parameters that determine cooked ham quality.

3.1. Colour

The colour of meat and meat products is an important quality attribute that influences consumer acceptance (Yildiz-Turp, Sengun et al. 2013). Colour belongs to appearance of food products, and in cooked ham it is possible to see a noticeable light pink colour as a consequence of nitrite addition (Toldrá, Mora et al. 2010).

The CIE *L* a* b** was developed in 1976 and provided uniform colour differences in relation to human perception. The CIELAB coordinates (*L* a* b*)* are directly read. It is considered the CIELAB uniform space in which two colours coordinates, *a** and *b**, as well as a psychometric index of lightness, *L**, were measured. The parameter *a** takes positive values for reddish colours and negative values for the greenish ones, whereas *b** takes positive values for yellowish colours and negative values for the bluish ones. *L** gives the measurement of luminosity, that can be considered as equivalent to a member of the greyscale, between black and white (Pathare, Opara et al. 2013).

In the last ten years, the CIE *L* a* b** was mostly used by many authors to measure colour in meat or meat products such as cooked ham (Utrera, Armenteros et al. 2012, Li, Szczepaniak et al. 2011, Valous, Mendoza et al. 2009, Válková, Saláková et al. 2007, Cheng and Sun 2007, Pipek, Rohlík et al. 2012, Tomović, Jokanović et al. 2013, Van de Perre, Ceustermans et al. 2010), cooked ham joints (Desmond, Kenny et al. 2002), hams (Person, McKenna et al. 2005, Schilling, Marriott et al. 2004), pork meat (Norman, Berg et al. 2003, Person, McKenna et al. 2005) and cooked turkey ham (Pedroso and Demiate 2008).

Lightness, *L** is considered by Garcia-Esteban, Ansorena et al. (2003) as the main parameter governing the quality of meat products. Dvorak, Musilova et al. (2001), studded methods for objective pork quality evaluation on a production line in a large slaughterhouse using colour measurements with CIE *L*a*b** system, and concluded that

*a** value was the most important aspect of colour, although its correlation coefficients with quality parameters such as pH and drip loss were very low. The colour co-ordinate *a** is also described as the most sensitive parameter of colour measurement, characterizing red colour and colour stability (Garcia-Esteban, Ansorena et al. 2003)

3.2. Texture

The composition and characteristics of the muscle influence texture of meat products (Utrera, Armenteros et al. 2012). Several texture parameters, such as hardness, elasticity, chewiness and viscosity enlarge as the fibre cross-sectional area and the peri- and endomysium thickness increase (Lachowicz, Sobczak et al. 2003). Concerning the texture of cooked hams it will also depend on several factors, such as, the extent of heating that causes structure breakdown, the moisture content, the extent of proteolysis as consequence of myofibrillar protein breakdown, the content of connective tissue and intramuscular fat. (Toldrá, Mora et al. (2010). Myosin and actin contribute to water-holding, emulsifying capacity, binding ability and gelation that occur during ham cooking (Asghar, Samejima, & Yasui, 1985, Pérez-Juan, Flores et al. (2008). Cooling methods affect the tenderness, juiciness, and overall texture (Desmond, Kenny et al. 2000).

There are two techniques widely used to evaluate texture in cooked ham, these are Texture Profile Analysis (TPA) and Warner-Bratzler Shear (WBS)

Texture profile analysis is one of the tests that attempt to imitate with instruments the conditions to which the food is subjected in the mouse or on the plate (Bourne 1978). TPA is carried out with a texturometer using a small plate-faced cylinder to compress a bite-size piece of food to 25% of its original height (75% compression) at least twice in a reciprocating motion and quantifying the mechanical parameters from the recorded force-deformation curves (Bourne 1978, Szczesniak 2002). From the analysis of solid and semisolid foods seven textural parameters could be obtained. Szczesniak (2002) and Bourne (1982) give an extensive description of each parameter in a physical and sensory view and Válková, Saláková et al. (2007) remark that the most desirable physical properties of a high quality cooked ham are cohesiveness, firmness and juiciness.

In the last years, many textural TPA works were done in meat products like cooked ham (Zell, Lyng et al. 2012, Utrera, Armenteros et al. 2012, González H, Suárez M et al. 2009a, González H, Suárez M et al. 2009b, Válková, Saláková et al. 2007, Cheng, Sun et al. 2005,

Cheng and Sun 2007, Desmond, Kenny et al. 2002) and turkey cooked ham (Pedroso and Demiate 2008).

The Warner-Bratzler Shear apparatus consists of a stainless steel blade (0.040 in. thick) with an equilateral triangle hole, forcing the meat into the V of the triangle until it is cut through. A force gauge measures the maximum force encountered during this action. Caine, Aalhus et al. (2003) explain that Warner-Bratzler Shear force is an imprecise predictor of beef tenderness but has become the most common method for evaluating beef tenderness. As same as TPA many works were carried out to evaluate texture in meats products like cooked ham (Tomović, Jokanović et al. 2013, Van de Perre, Ceustermans et al. 2010, Válková, Saláková et al. 2007, Cheng, Sun et al. 2005, Pipek, Rohlík et al. 2012), pork loin muscle (Liu, Xiong et al. 2011, Christensen, Ertbjerg et al. 2011, Norman, Berg et al. 2003), lamb muscle (Han, Morton et al. 2009), ham (Valous, Mendoza et al. 2009) and beef muscles (Sullivan and Calkins 2010).

Only as example mode and in a different meat product Caine, Aalhus et al. (2003) found that TPA explained slightly more of the variation in sensory panel characteristics of beef tenderness than WBS.

3.3. Proteolysis

During processing of meat products muscle proteolysis occurs due to degradation of both sarcoplasmic and myofibrillar proteins by muscle endopeptidases or by exopeptidases (Sentandreu and Toldra 2001). The main final products of proteolysis are small peptides and free amino acids that can contribute to an adequate taste, but an excessive protein breakdown may be undesirable, resulting in an excessive softening of the meat product. In other cases, an excessive accumulation of peptides and free amino acids may result in strange tastes, for example, bitter or metallic tastes (Fidel, María-Concepción et al. 2008)

Myofibrillar proteins and collagen account for the hardness of muscle fibres and perform contractile, regulatory and cytoskeletal functions. Sarcoplasmic proteins perform biochemical functions (Soriano Pérez, García Ruiz et al. 2003). Thus, meat proteins are extracted by two different methods (Fidel, María-Concepción et al. 2008), sarcoplasmic proteins are water soluble and are extracted using low ionic solutions (Pioselli, Paredi et al. 2011) such as phosphate buffers (pH 6,5-7,6) (Chaves-López, Paparella et al. 2011, Dai, Miao et al. 2013, Pérez-Juan, Flores et al. 2008, Patel, Solomon et al. 2006) with low

concentrations (0,01 M / 0,1 M) at a dilution ratio of 1:10 (Fidel, María-Concepción et al. 2008). After centrifugation sarcoplasmic protein are in the supertant and the precipitate, contains the myofobrillar proteins that are resuspended at a dilution of 1:10 in 0.1 M phosphate buffer, pH 6.5, containing 0.7 M KI (Fidel, María-Concepción et al. 2008) because myofibrils are soluble in high ionic medium (Pérez-Juan, Flores et al. 2008).

Proteolysis can be reliably evaluated by SDS-PAGE or by chromatographic methods. SDS-PAGE involves dissolution of sarcoplasmic and myofibrillar proteins with sodium dodecyl sulfate under heating (Hughes, Kerry et al. 2002). The percentage of polyacrylamide in the resolving gel depends on the target proteins that are being analysed. Proteins can be visualized as bands by staining with Coomassie Brilliant Blue R-250 or with silver when more sensitivity is needed. The gel can be scanned for quantitative purposes so that the peak area for each respective band is calculated and compared with those from standards (Fidel, María-Concepción et al. 2008).

Reverse phase high performance liquid chromatography (RP-HPLC) is commonly used to follow proteolysis, evaluating peptide formation and protein hydrolysis and many works were done using this method in different foods such as human milk (Santos and Ferreira 2007), cheese (Ferreira, Veiros et al. 2006) and malt and beer (Silva, Nogueira et al. 2008).

Concerning free amino acids, analyses, the most used technique is HPLC using reverse phase chromatography or cation exchange with post-column derivatization (Rigas 2012). For solid samples, extraction and precipitation of proteins and lipids is needed and samples must be deproteinized (e.g., acetonitrile or ethanol give good results) and then analysed (Fidel, María-Concepción et al. 2008). Amino acids must be derivatized, due to the lack of cromofores, different derivatization reagents can be used; phenylthiocarbamyl derivatives are preferred for meat amino acid analysis. The derivatized amino acids are separated through the reverse-phase column, followed by UV detection at 254 nm.

Pre-column derivatization is frequently applied, however according to Fidel, María-Concepción et al. (2008) in the case of using a cation-exchange column, nonderivatized amino acids are separated and then derivatized by postcolumn derivatization is with ninhydrin or *o*-phthaldialdehyde (OPA) before the UV or fluorescence detection.

Another technique widely used to determine free amino acids is Gas Chromatography with Mass Spectrophotometry. Due to the low volatility of amino acids previous derivatization is required (Otter 2012). Determinations of amino acids by GC-MS with only one step of derivatization (Jiménez-Martín, Ruiz et al. 2012) is an alternative technique for amino acid

determinations. This derivatization step could be carried out, as Pérez-Palacios, Melo et al. (2013) explain, with bis-trimethyl silyl trifluoro acetamide (BSTFA) or N-Methyl-N-(tertbutyldimethylsilyl) trifluoroacetamide (MTBSTFA), but the last derivatizing agent produce more stable compounds against different reactions such as hydrogenolysis, hydrolysis, mild reaction and oxidation being more appropriate to GC-MS.

3.4. Volatile compounds

Aroma is an important parameter for the choice of a product by consumers (Chiesa, Soncin et al. 2006) and results from the volatile compounds generated during cooked ham processing, due to biochemical changes, that are a consequence of enzymatic reactions. Proteolysis, lipolysis and compounds that arise from enzymatic reactions will act as substrate in further chemical reactions, namely, Maillard and Stecker reactions (Toldrá, Mora et al. 2010).

Extraction of volatile compounds can be performed by flow-through and batch equilibrium or pre-equilibrium techniques (Jeleń, Majcher et al. 2012, Chiesa, Soncin et al. 2006). The first include exhaustive techniques such as purge and trap, sorbent trap, and solid phase extraction (SPE). The second includes exhaustive liquid-liquid extraction, soxhlet or sorbent extraction. Non-exhaustive techniques include solid phase microextraction (SPME). Other methods used for flavour isolation include high vacuum transfer (HVT), solvent assisted flavour evaporation (SAFE), liquid/liquid extraction (LLE), simultaneous distillation/extraction (SDE) among others (Jeleń, Majcher et al. 2012, Chiesa, Soncin et al. 2006).

Headspace gas chromatography (HS-GC) is the combination of headspace sampling and gas chromatography. It has been routinely employed for analysis of volatile compounds from liquid and solid samples (Yuwen, McCaffrey et al. 2008).

Static headspace extraction and HS-SPME is based on the partition of analytes and is a non-exhaustive extraction. The technique of SPME is based on the partition of the analyte between the sample matrix and a stationary phase that is a fibre coated with an extracting liquid (polymer) or solid (sorbent) phase. Equilibrium is reached between the concentration of the analyte in sample and the amount of analyte sorbed on the fiber, depending on the distribution coefficient. It is a solvent-free technique with low cost. Different absorption

fibers are available (PDMS, CW, PA) as well as fiber coatings in which adsorption processes dominate (DVB/PDMS, Carboxen/PDMS) (Jeleń, Majcher et al. 2012).

HS-SPME GC-MS was applied in the analyses of different kinds of meat or meat products (Gianelli, Salazar et al. 2012) such as cooked ham (Leroy, Vasilopoulos et al. 2009, Chiesa, Soncin et al. 2006, Comi and Iacumin 2012), dry-cured ham (Gianelli, Flores et al. 2002), beef meat(Tomović, Jokanović et al. 2013), chicken meat(Mariutti, Nogueira et al. 2009), fish meat (Giri, Osako et al. 2010, Miyasaki, Hamaguchi et al. 2011), pork meat (Elmore, Mottram et al. 2001) and fermented sausages (Olivares, Navarro et al. 2011).

Chapter 2. Materials and methods

4. Spent *Saccharomyces* yeast extract

Brewing spent yeast biomass was supplied by a local beer industry. The yeast rejected by the brewing industry was collected to transparent glass bottles with 1 h capacity and stored at 4°C.

The brewing spent yeast biomass was washed at least three times with deionized water at a ratio of 1:3 (w/v) (yeast biomass : water), between each wash it was centrifuged at 10,000*g*, 4°C, 5 min. The cell wall was destroyed by a mechanical process using glass beads at a ratio of 1:2:1 (biomass: acetate buffer 0.04 M, pH 5: glass beads, g/v/g) by vortexing 10 times (1 min each), with 1-min cooling intervals on ice. After removing the glass beads by allowing the suspension to stand, the homogenate was centrifuged at 15,000*g*, 4°C for 30 min. The resulting pellet that contained cell wall was discarded and the supernatant was used as spent yeast extract.

For each batch of cooked ham production, one litter of spent yeast extract prepared as described previously was lyophilized. After 48 hs of lyophilisation, samples were re-suspended with 0.04 M acetate buffer to achieve a final volume of 250 ml. Proteolytic activity was evaluated according to Cupp-Enyard method.

5. Sampling

5.1. Conventional cooked ham

Conventional cooked ham samples were prepared by the industry at pilot scale using 20kg of pork leg cuts that were injected with 35% brine. Samples were tumbled with 7 cycles/min, at controlled temperature between 0-4ºC during 18 hours under vacuum, to avoid further undesired oxidations and improve salt diffusion. Meat cuts were packaged to form cooked ham pieces with approximately 900 g each. Sixteen samples were cooked in an oven with a constant atmosphere temperature of 80ºC to obtain a central temperature of 68ºC. Cooking time was 1 hour and 30 min. Flow diagram is shown in Figure 2.

After the cooking stage, samples were cooled with ice and refrigerated at 0-4ºC overnight. Samples were refrigerated and stored during 12 days for maturation as it is usually done by

the industry. Two samples were randomly collected to perform laboratorial analysis (n= 2) and another 2 samples randomly collected (n= 2) after 90 days to repeat laboratorial analyses and evaluate changes that occurred during shelf-life. Two batches were produced, thus, a total of 4 hams were analysed at 12 days and 4 hams were analysed at 90 days.

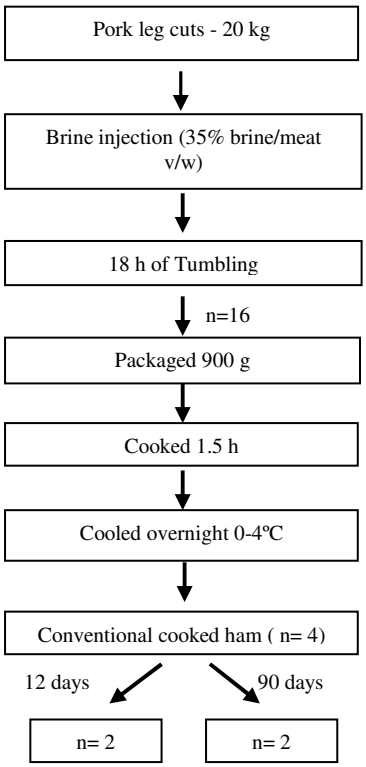

Figure 2: Flow diagram of each batch of conventional cooked ham production

5.2. Cooked hams with reduced tumbling time

Several cooked hams with reduced time of tumbling were prepared including single addition of 0.04 M acetate buffer used to prepare spent yeast extract (control) and addition of spent yeast extract dissolved in 0.04 M acetate buffer and different cooking times. Figure 3 shows the sampling of two different types of cooked ham with reduced tumbling time produced by the industry at pilot scale. Two portions of 20 kg of pork leg cuts were injected with 35% of brine. Before tumbling one portion was added with 1% acetate buffer 0.04 M, whereas the other portion was added with 1% spent yeast extract dissolved in acetate buffer 0.04 M. The two portions were tumbled separately 9 h at 0-4ºC with vacuum. After tumbling, 32 hams were packaged as performed with conventional hams.

Cooking step was performed at four different cooking times, 1.5, 2, 2.5 and 3 hours. After the cooking stage, samples were cooled with ice and refrigerated at 0-4ºC overnight. Samples were stored during 12 days for maturation as usually done by the industry.

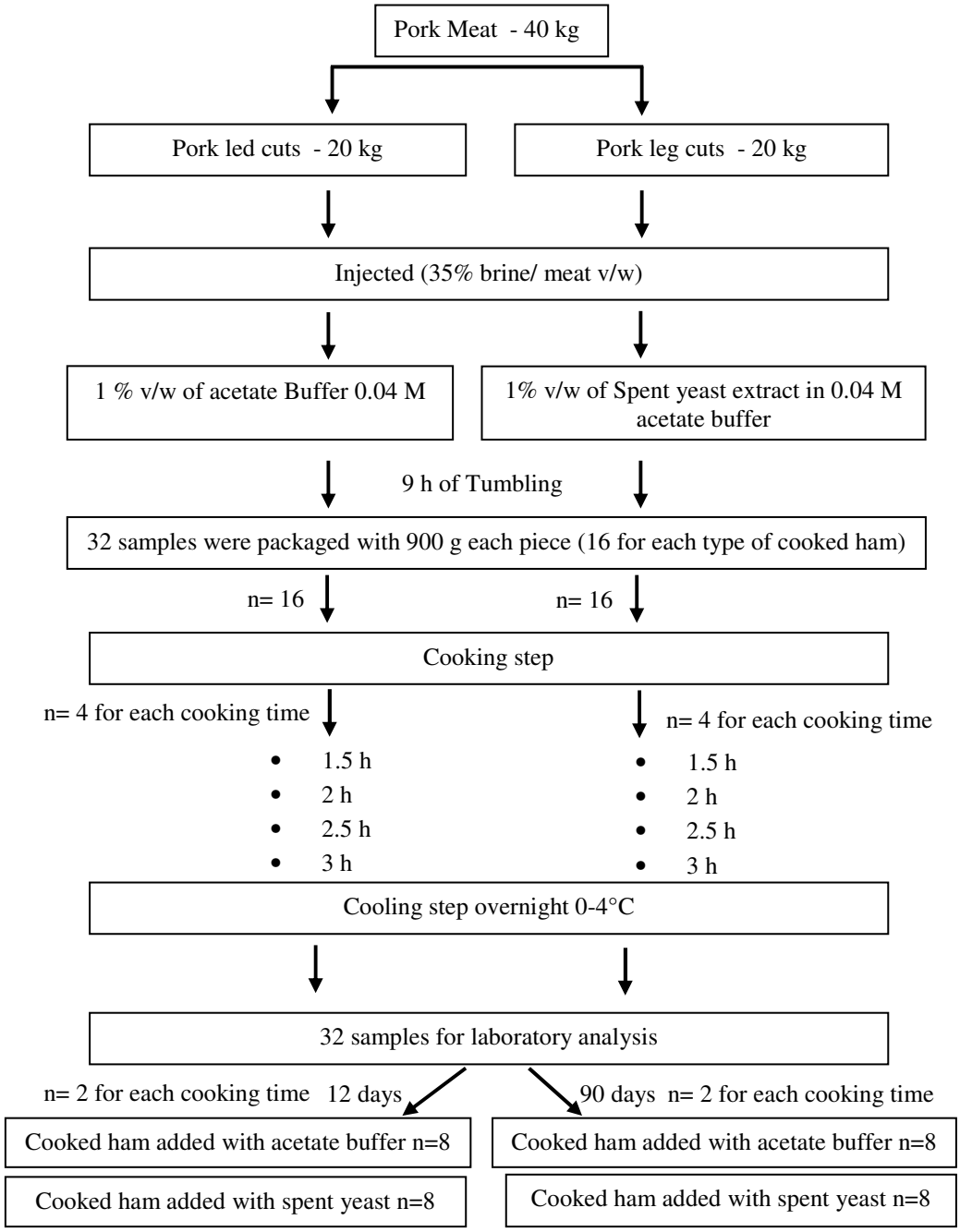

Figure 3: Flow diagram of the two portions of cooked ham with reduced tumbling time, representing the sampling of cooked ham with 0.04 M acetate buffer and cooked ham with spent yeast in 0.04 M acetate buffer produced at pilot scale.

Two samples of each cooking time were collected to perform laboratorial analysis (n= 8) and another 2 samples of each cooking time were collected (n= 8) after 90 days to repeat

laboratorial analyses and evaluate changes that occurred during shelf-life. All this process was repeated twice, thus two batches were prepared repeating the procedure described previously a total of 64 ham samples were analysed.

5.3. Cooked hams for Sensory analysis

Sensory analysis was carried out in three batches of cooked ham produced by the industry at pilot scale: one batch of conventional cooked ham, another batch of cooked ham with reduced tumbling time added of 1% 0.04 M acetate buffer and the last batch also with reducing tumbling time and added with 1% spent yeast extract prepared with 0.04 M acetate buffer. All samples were cooked during 1.5 h. Sensory analyses were performed at the industry after 12 and 90 days of maturation by a trained panel composed of 13 panellists.

6. Analytical methods for evaluation of cooked ham composition

6.1. Proximate composition

Proximate composition, moisture, ash, protein and fat content were evaluated using AOAC (2000) methods (No. 950.46, No. 920.153, No. 928.08 and No. 991.36, respectively). The nitrogen to protein conversion factor was 6.25. Total Na^+ and K^+ content was determined by titration using the method of Vieira, Soares et al. (2012). Samples were defrosted at room temperature and homogenated. After homogenization, 2 g of each cooked ham were diluted with 20 ml of distillate water and mixed with Ultra Turrax blender (completed to a final volume of 40 ml) and after that Na+ and K+ in the cooked ham samples were quantified by flame photometry (flame photometer model PFP7, JenWay®, England).

Analyses of pH were performed according to Liu, Xiong et al. (2011) and Samelis, Kakouri et al. (1998), 3 g of ham were blended with 30 ml of distilled water, the mixture was homogenized with an Ultraturrax T25 Homogenizer (Jankel & Kunkel, Lisboa, Portugal) during 20 s. The pH of the homogenate was measured with a combined pH glass electrode connected to a pH-meter (MicropH 2001, Crison, Barcelona, Spain). All determinations were carried out in duplicated to obtain mean values.

6.2. Colour Analysis

Colour analysis was performed using a Minolta colorimeter (Model No. CR-400, Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA) calibrated for internal light (D65) before the measurement of *L** (lightness), *a** (redness/greenness) and *b** (yellowness/blueness). Hams were cut on the middle of the piece and measurements were carried out at three randomly chosen points.

6.3. Texture Profile Analysis (TPA)

TPA analysis was performed according with González H, Suárez M et al. (2009a). Cooked ham samples were cut into cubes 20 x 30 mm using a steel cutter from a 30 mm central slice (Figure 4). Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems®) equipped with a load cell of 25 kg and a probe of 20 mm diameter SMSP/20. The operating conditions were: pre-test speed of 2 mm s^{-1}, test speed of 10 mm/s, post-test speed 5 mm s^{-1} compression time between 1 sec. Ham samples were uniaxially compressed by 40% of the original height.

Figure 4: Sample cubes for TPA analysis obtained from central slice of each cooked ham

6.4. Proteins and peptides analysis by RP-HPLC/UV

The extraction of cooked ham proteins was carried out according to Fidel, María-Concepción et al. (2008). Briefly, 3 g of minced cooked ham was suspended in 30 ml on 0.03 M phosphate buffer, pH 6.5. The suspension was vortered during 4 min and then centrifuged at 10,000*g* for 20 min at 4°C. The supernatant containing the proteins soluble in diluted phosphate buffer 0.03 M, mainly Sarcoplasmic proteins (PSDB) was collected and the pellet was washed twice with phosphate buffer in order to recover all soluble proteins that were removed by the buffer. Then, the remaining pellet was weighed and re-suspended at a dilution of 1:10 in 0.1 M phosphate buffer, pH 6.5, containing 0.7 M KI to extract the proteins soluble in strong ionic buffer (PSSIB) mainly, myofibrillar proteins. The suspension was homogenized and centrifuged at 10,000*g* for 20 min at 4°C. Samples were studied in duplicated. Additionally, raw pork meat was also analysed using the same procedure described above, to obtain the RP-HPLC of native pork meat proteins.

The sample extracts were filtrated trough 13 mm and 0.45 µm cellulose filter (Teknokroman) and were analysed according to Silva, Nogueira et al. (2008). The reversed-phase chromatographic analysis was carried out using an analytical HPLC system (Jasco, Tokyo, Japan) equipped with a quaternary low pressure gradient HPLC pump (Jasco PU-1580), a degasification unit (Jasco DG-1580-53 3-line degasser), an autosampler (Jasco AS-2057-PLUS), a MD-910 Multiwavelengh detector (Jasco) and a 7125 Rheodyne injector valve (California, USA) with a 100 µl loop. The column was a Chrompack P 300 RP (polystyrenedivinylbenzene copolymer, 8 µm, 300Å, 150 x 4.6 mm i.d.) (Chrompack, Middleburg, The Netherlands) separation was performed at room temperature with a flow rate of 1 ml/min. Gradient elution was performed with two eluents: (A) 0.1% TFA in water (TFA, Aldrich Chemicals Co., Milwaukee, WI) and (B) 0.1% TFA in acetonitrile (EM scientiRc, Gibbstown, NJ). Gradient elution was carried out with 5% B for 0-5 min, 10% B for 5-10 min, 55% B for 10-30 min and 5% B for 30-45 min. The eluted peaks were monitored at 214 nm and data acquisition was accomplished using the Borwin Controller Software, version 1.50 (JMBS Developments, Le Fontanil, France).

6.5. Free Amino Acids Analysis

Free amino acids were analysed according to Pérez-Palacios, Melo et al. (2013). Ham samples (1 g) were weighed into a 15 ml flat-bottom glass vial containing a stir bar, and 7.5 ml of 0.1 N HCl were added. The vial was sealed with a screw-top cap and stirred at high speed 30 min at 40°C. The mixture was then centrifuged (5,000 rpm, 30 min), and the supernatant was transferred to a 15 ml graduated vial, completed until 10 ml with distilled water and centrifuged again (10,000 rpm, 15 min). Afterwards 100 µl of the extract, 250 µl of acetonitrile were added for protein precipitation. Tubes were centrifuged at 8,000*g* for 5 min. From this point, standard solutions and food samples followed the same process. Then, 100 µl of the supernatant (or the standard solution) were transferred to heat-resistant tubes, and 100 µl of internal standard Norleucine solution (5 µg ml^{-1}) were added. Tubes were dried under nitrogen. 50 µl of dichloromethane were added to the dried samples, and again evaporated under nitrogen. Finally, 50 µl of the derivatization agent N-methyl-N-tert-butyldimethylsilyl- trifluoroacetamide (MTBSTFA) and 50 µl of acetonitrile were added to the dried tubes, which were shaken and subsequently incubated at 100°C for 60 min.

The chromatographic analysis was carried out in an Agilent 6890 gas chromatograph (Agilent, Avondale, PA, USA) coupled to a MS detector (Agilent 5973). The MS system was routinely set in selective ion monitoring (SIM) mode and each compound was quantified based on peak area using one target and one or two qualifier ions.

A portion of 1 µl of the derivatized extract was injected in splitless mode onto the column. The column used was a 58 m×0.32 mm i.d., 0.05 µm, HP-5 (Hewlett-Packard), with a 5 % phenyl–methyl polysiloxane bonded phase fused silica capillary column. Column head pressure was 12.8 psi, resulting in a flow of 1.2 ml min^{-1} at 280°C. The oven program was as follows: 170°C for 5 min, 4°C min^{-1} ramp to 200°C, held at 200°C for 3 min, 4°C min^{-1} ramp to 290°C, held at 290°C for 16 min. Mass spectra were obtained by electronic impact at 70 eV, with a multiplier voltage of 2,056 V. The transfer line to the mass spectrometer program was as follows: 280°C for 35 min, 0.5°C min^{-1} ramp to 290°C, collecting data over the m/z range 30–600. Total run time was 55.75 min. Free AA were identified from Nist 98 data bank (NIST/EPA/NISH Mass Spectral Library, version 1.6, U.S.A.) and using both their retention time and by comparison of their characteristic m/z ions.

FAA quantification in cooked ham samples was carried out in the SIM mode by external calibration curve method. For each AA, a calibration curve (quantification ion AA peak area/quantification ion IS peak area versus AA amount) was constructed, obtaining R^2 values of 0.99. The final results, expressed in microgram per 100 gram dry weight, take into account the moisture content and the exact weight of the sample. Determination of FAA was done in duplicated.

6.6. Volatile Compound analysis

Volatile compounds were extracted by headspace solid phase microextraction (HS-SPME) and analysed by Chromatographic analysis (Varian 4000 GC-MS-MS) coupled to a mass selective detector (Varian 240MS/4000 Mass Spec). The optimised HS-SPME procedure used for evaluation of volatile compounds in cooked ham samples was adapted from Pérez-Palacios, Petisca et al. (2012) with slight modifications: sample (1 g), was transferred to a 20 ml vial, containing 2 ml of water and 1.2 g of NaCl. 20 µl of L-Linalool (10 µg ml^{-1}) was added as internal standard, and the vial was immediately sealed at once and kept at 4ºC until analysis, in order to avoid losses due to the high volatility of the compounds under study. Duplicate vials were prepared for each ham sample. To extract volatile compounds a PDMS-DVB SPME fibre (65 µm thicknesses, Supelco Co., Bellefonte, PA, USA) was used with CombiPal sampler. The agitation temperature was 55±1ºC and each sample was incubated during 5 min and agitation speed used was 500 rpm. Fibre was exposed to the HS for 45 min under constant agitation (250 rpm). Thereafter, the SPME fibre was inserted and desorbed for 4 min at 230ºC, in the split-less mode, with 1 ml min^{-1} flow.

Volatiles were separated on a 5% diphenyl/95% dimethylpolysiloxane (BR-5ms) bonded phase fused-silica capillary column (Bruker; 30 m x 250 µm i.d., film thickness 250 µm), operating at 18.8 psi column head pressure, resulting in a flow of 1 ml min^{-1} at 40ºC. The oven temperature programme was isothermal for 1 min at 40ºC, raised to 250ºC at a rate of 25ºC min^{-1} and then raised to 300ºC at 5ºC min^{-1}. The transfer line to the mass spectrometer was maintained at 280ºC. Mass spectra were obtained by electronic impact at 70 eV, with a multiplier voltage of 2,056 V, collecting data at a rate of 1 scan s1 over the m/z range 30–500. Volatile compounds were identified by comparison with the mass spectrum from standards and Nist 2005 data bank (NIST/ EPA/NISH Mass Spectral Library, version 2.0, U.S.A.). The volatile compounds were also detected by its

characteristic ions. Fibre blanks were run daily to ensure the absence of contaminants or carry-over.

Figure 5: Samples preparation for volatile compounds analysis

6.7. Sensory analysis of cooked ham

Each sample of cooked ham was coded using three digits. Evaluated parameters were texture in mouth, colour, flavour and global appreciation (Figure 6). A 6 points structured scale was applied, score 1 for bad, score 2 not satisfy, score 3 satisfy, score 4 good, score 5 very good and score 6 excellent. The prove sheet included a space for observations and at the end asked the panellists which samples they prefer. It was recommended to panellists that they should take a pause between each samples prove, they should also clean the mouth with water, chew a small cracker and wait at least 1 minute to ensure that all flavours from the previous sample have disappear. Sensory analysis was performed by 11 trained panellists at the industry.

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Sensory Analysis - sheet tester

Name

Metric:
1 = Bad
2 = Not satisfy
3 = Satisfy
4 = Good
5 = Very good
6 = Excellent

between each sample make a pause, washing your mouth with water, chewing a little water-salt biscuit and waiting at least 1 minute to ensure that the all flavours from the previous sample disappear of

Compare the products 834, 421 and 578, giving a score from 1 to 6, for each analyzed variable. At the end, highlight which is the sample that you prefer.

	834	421	578
Texture (in mouth)			
Colour			
Flavour			
Global Apreciation			
Observations			

Which samples do you prefer? _____

Figure 6: Example of the sensory sheet given to panellists to carry out sensory analysis of cooked hams

7. Analytical methods for evaluation of spent yeast extracts

7.1. Proteolytic activity of spent yeast extracts

Proteolytic activity of spent yeast extracts that was previously lyophilized and re-suspended in 0.04 M acetate buffer was performed according to Cupp-Enyard (2008) method with slight modifications. Five ml of 0.65% casein were placed in 15 ml vials and equilibrated during 5 min at 37ºC. Spent yeast extract (0.7 ml) were added and incubated during 37ºC to promoted casein hydrolysis. Reaction was stopped adding 5 ml of 110 mM trichloroacetic acid solution. Additionally, 10 mM sodium acetate buffer with 5 mM calcium acetate pH 7.5 were added to ensure a final equal volume in every tube. Then tubes were incubated during 30 min at 37ºC. After incubation step, tubes were centrifuged at 10,000*g* during 5 min at 4ºC to remove the casein that was not hydrolysed. Next, 2 ml of supernatant were transferred to another 10 ml graduated tube and was added 5 ml of 500 mM sodium carbonate solution and 1 ml of Folin & Ciolcaltea's 0.5 mM reagent, mixed and incubated at 37ºC during 30 min immediately. Blank control was prepared as same as

samples tubes but without enzyme and the addition of 1ml of 10 mM sodium acetate buffer with 5mM calcium acetate pH 7.5. After incubation, the absorbance of the samples was measured by a spectrophotometer using a wavelength of 660 nm. A calibration curve was done with 1.1 mM L-tyrosine as standard with crescent addition in six point except the blank tube (0.05, 0.10, 0.20, 0.40, 0.50 ml, respectively) and purified water was added until a final volume of 2 ml. Results were expressed in Units ml^{-1} calculated by this equation:

$$Units/ml = \frac{(\mu mol\ \text{tyrosine equivalent release}) \times \text{Total assay volume (ml)}}{\text{Enzyme volume (ml)} \times \text{Assay time (min)} \times \text{Volume for colorimetric determination (ml)}}$$

Where the number of micromoles tyrosine equivalents released was obtained from the slope equation and the total volume of the assay was 11 ml. This result was divided by three other quantities: the time of the assay, which was 10 minutes, the volume of enzyme used in the assay, which was varied and the volume of millilitres used in colorimetric determination, which was 2 ml.

7.2. Protein

Total protein in spent *Saccharomyces* extract was measured by Bradford micro assay method at 595 nm. 5µl of samples was added in at least 3 micro plate wells to have representative reads and 150 µl of Sigma-Aldrich Bradford reagent. Separately, blank control was done replacing sample by 5 µl of water and a calibration curve was performed with BSA (Bovine Serum Albumin) 2 mg ml^{-1} and points were from this solution at 0.1-1.4 mg ml^{-1} of protein.

8. Statistics analysis

SPSS version 22 was used to perform statistical treatment of results. To evaluate the modifications in cooked hams at the beginning and end of shelf life a *t*-test was performed for each type of cooked ham and in the case of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract the *t*-Test was applied for each cooking time at the beginning and end of shelf life. A two-way analysis of variance was

performed to establish the effect of yeast addition and the effect of cooking time on the proximate composition, CIE *Lab* attributes, TPA analysis, proteolysis profile and volatile compounds in cooked ham added with acetate buffer (control) and cooked ham added with spent yeast extract. Cluster analyses were performed using free amino acids and volatile compounds as variables. PCA (Principal Component Analyses) was done with variables that were significantly different due to the effect of yeast extract addition or due to shelf life modifications to reduce the dimensionality of data and highlight most relevant conclusions of the work.

Chapter 3. Results and Discussions

Section A: Conventional cooked ham: Characterization of its quality attributes at 12 and 90 days

9. Quality attributes of conventional cooked ham and its stability

9.1. Conventional cooked ham at 12 days

9.1.1. Proximate composition

Proximate composition of conventional cooked ham is reported in Figure 7. Moisture ranged from 75.21 to 75.97%. Protein, fat and ash percentages varied from 15.40 to 16.40 %; 1.90 to 4.10 % and 3.40 to 4.10 %, respectively. Mean pH value of conventional cooked ham was 6.4 which is above the one reported by Tomović, Jokanović et al. (2013) and Li, Szczepaniak et al. (2011) that mention pH values of 6.25 and 6.2, respectively.

Sodium content ranged between 0.68 to 0.70 g/100 g and potassium varied between 0.33 and 0.37 g/100 g of cooked ham. Na^+ values were lower than those reported by other authors that describe mean values of 2.79 g/100 g (Válková, Saláková et al. 2007) and 2.1 g/100 g (Casiraghi, Alamprese et al. 2007) . K^+ values were also lower than those described by Cardoso, Henry et al. 2012 who reported that cooked ham had K^+ contained 0.42 g/100 g.

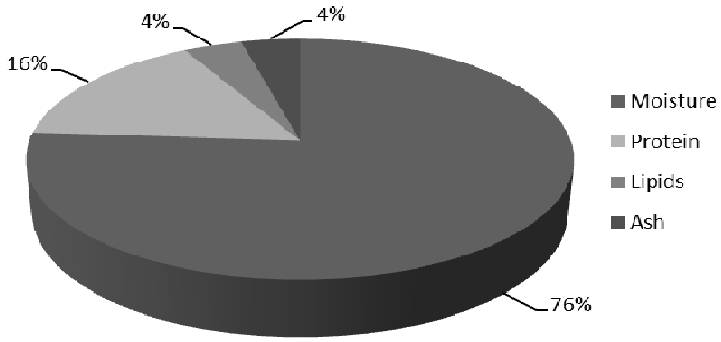

Figure 7: Proximate composition (% w/w) of Conventional cooked hams

According to NP 4393-2001 the ratio moisture/protein should be ≤ 4.2 for this kind of meat product. Conventional cooked ham samples presented an average moisture value of 75.8% and total proteins value of 15.8%, the relation moisture/total proteins was 4.8, being higher than that suggested by Portuguese recommendations. According to the same standard the minimum content of total protein for "fiambre da perna" is 14%, the samples satisfy this

requirement. Additionally, the moisture content is in agreement with other works from literature that studied cooked ham composition (Tomović, Jokanović et al. 2013, Moretti, Bellagamba et al. 2009). Similar values were observed for total protein (Zell, Lyng et al. 2012), fat (Válková, Saláková et al. 2007) and ash content (Casiraghi, Alamprese et al. 2007).

9.1.2. Colour and texture parameters

Mean CIE *L** value ranged from 57.5 to 65.1, *a** value varied from 9.3 to 14.9 and *b** value from 7.3 to 8.1. Colour and texture parameters of conventional cooked ham samples analysed 12 days after production are presented in Table 2. According to Tomović, Jokanović et al. (2013) in cooked hams, colour is one of the most important quality characteristics. The mean CIE *Lab* values *L** and *b** of conventional cooked ham were similar to those described by Li, Szczepaniak et al. (2011), Moretti, Bellagamba et al. (2009). However, *a** value was lower than the ones obtained by authors cited before; this could be justified by the lower myoglobin concentration in muscle and different concentration of nitrite in brine solution.

Table 2: Mean colour attributes and texture profile analysis parameters of conventional cooked hams (results are expressed as mean ± SD)

Parameter	**Value** (n= 4)
*L**	62.3 ± 3.03
*a**	11.8 ± 1.60
*b**	7.79 ± 0.30
Hardness (N)	51.7 ± 8.29
Springiness (mm)	1.35 ± 0.08
Cohesiveness (-)	0.72 ± 0.05
Gumminess (N)	37.1 ± 4.76
Chewiness (N.mm)	42.7 ± 12.0

Concerning texture parameters comparison with literature is difficult because experimental conditions for TPA analyses are variable. Additionally, it is expected that TPA values differ due to the different raw materials and not standardized production systems. TPA Hardness parameter was almost three times higher than that described by Válková, Saláková et al. (2007) and Utrera, Armenteros et al. (2012) and the same was observed for Chewiness and Gumminess.

9.1.3. Proteolysis and peptides formation

Meat proteolysis and peptide formation were evaluated by RP-HPLC. Two different extraction methods were applied, one for extraction of soluble proteins and peptides in diluted buffer (PSDB) and the other for extraction of proteins and peptides soluble in strong ionic buffer (PSSIB). Figure 8 presents the typical chromatograms obtained for raw pork meat. The chromatograms of PSDB and PSSIB protein fractions of raw pork meat, presented a main peak between R_T 25 to 33 min corresponding to native intact meat proteins.

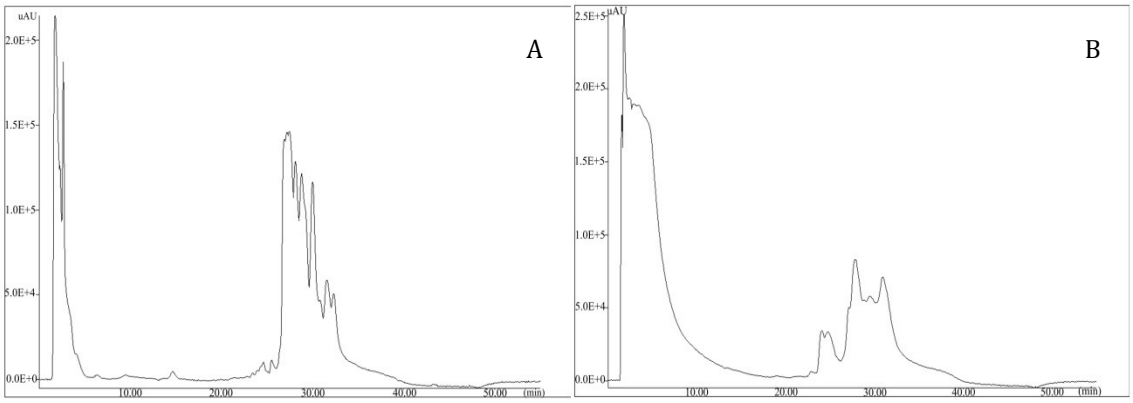

Figure 8: Typical chromatogram by RP-HPLC of raw pork meat. A: PSDB; B: PSSIB protein fractions

Typical chromatograms of PSDB protein fraction extracted from cooked hams analysed 12 days after production show three main peaks, one with R_T from 5 to 15 min, the second with R_T from 15 to 25 min and the third with R_T from 25 to 33 min (Figure 9).

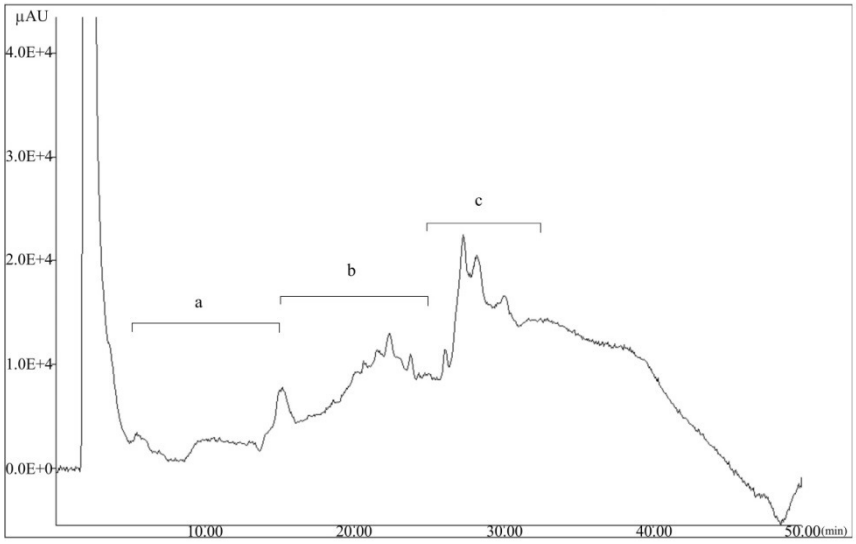

Figure 9: Chromatographic profile of PSDB proteins fraction by RP-HPLC of conventional cooked ham. a: R_T 5-15 min; b: R_T 15-25 min; c: R_T 25 to 33 min

Relative peak areas (RA) of raw pork meat and conventional cooked ham analysed 12 days after production are shown in Table 3. Peaks with T_R 5-15 min (13.2%) belong to polypeptides with molecular weight <14 kDa and peaks from R_T 15-25 min (50.7%) correspond to polypeptides and proteins >14kDa and < 60KDa. Peaks between T_R 25 to 33 min (36.1%) are from proteins >60 kDa. These limits were established by comparison with molecular weight standards from 14 to 97 kDa separated by RP-HPLC. The major peak areas in cooked ham profile are from R_T 15 to 25 min. A notorious degradation of native proteins of raw pork meat is observed in cooked ham samples as shown in Table 3.

Table 3: Quantification of PSDB protein fraction expressed as relative % of peak areas (RA) of pork meat and conventional cooked ham

	PSDB protein fraction*	
R_T (min)	Raw pork meat	Cooked ham
5-15 min	0.7%	13.2%
15-25 min	1.3%	50.7%
25-33 min	98%	36.1%

*RA (relative peak area expressed as percentage of total peak area) Samples n=4

PSSIB protein fraction of cooked ham was also separated by RP-HPLC (Figure 10). Chromatograms can be divided in two main peaks, one between R_T 15 to 25 min and another between R_T 25 to 33 min. Low molecular peptides and amino acids were eluted with solvent line at R_T <12 min.

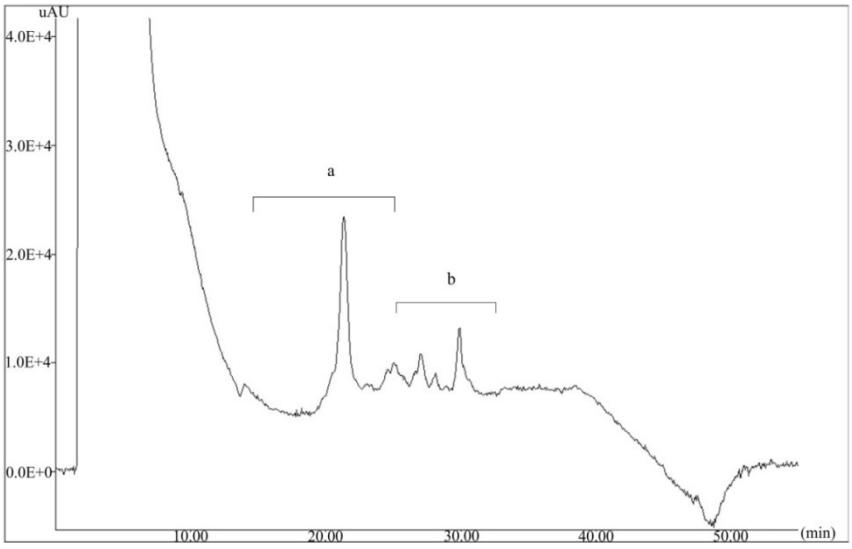

Figure 10: Chromatographic profile of PSSIB protein fraction by RP-HPLC of conventional cooked ham. a: R_T 15-25 min; b: R_T 25-33 min

The relative areas of PSSIB protein fraction of raw pork meat and cooked ham are summarized in Table 4. Differences were observed in the protein and polypeptide peak areas of pork meat and cooked ham. Major peak area of cooked ham is at R_T 15-25 min corresponding to polypeptides and proteins with molecular weight >14 kDa and < 60 kDa.

Table 4: Quantification of PSSIB protein fraction expressed as relative % of peak areas (RA) of pork meat and conventional cooked hams

	PSSIB*	
R_T **(min)**	**Raw pork meat**	**Cooked ham**
15-25 min	13.9%	63.6%
25-33 min	86.1%	36.4%

*RA (relative peak area expressed as percentage of total peak area). Samples n=4

The distribution of the two main peaks from PSSIB protein fraction can be explained by the proteolysis suffered from meat proteins during cooked ham production and due to phosphates action, proteolysis and heat effects. Enzymatic proteolysis could occur before cooking due to endogenous enzymes, however when 68-70ºC is reached enzymes are inactivated owing to the heat effects (Toldrá, Mora et al. 2010).

9.1.4. Quantification of free amino acids (FAA)

Calibration curve parameters are summarized in Table 5. To examine the performance of the proposed methodology, quality parameters for each individual AA were determined. Good linearity was obtained for the range 0.25 – 25 µg ml^{-1} for all AA with the exception of Isoleucine (0.25-50 µg ml^{-1}), with correlation coefficients higher than 0.99. LODs and LOQs of the analytical procedure were 0.08 µg μl^{-1} and 0.25 µg μl^{-1}, respectively.

Table 5: Calibration curves parameters and linearity range used for quantification of FAA in cooked hams

Peak number	Amino acid	LR (µg ml^{-1})	b	a
1	Alanine (Ala)	0.25-25	0.03840	-0.01300
2	Glycine (Gly)	0.25-25	0.04590	-0.00420
3	Valine (Val)	0.25-25	0.02560	-0.00630
4	Leucine (Leu)	0.25-25	0.05020	-0.00260
5	Isoleucine (Ile)	0.25-50	0.03160	-0.03480
6	Proline (Pro)	0.25-25	0.07460	-0.02930
7	Phenylalanine (Phe)	0.25-25	0.00260	-0.00060
8	Methionine (Met)	0.25-25	0.01740	-0.00380
9	Serine (Ser)	0.25-25	0.01850	-0.00242
10	Threonine (Thr)	0.25-25	0.00710	-0.00120
11	Aspartic acid (Asp)	0.25-25	0.02340	-0.00640
12	Hydroxyproline (Hyp)	0.25-25	0.00810	-0.00450
13	Cysteine (Cys)	0.25-25	0.00070	0.00010
14	Glutamic acid (Glu)	0.25-25	0.00007	-0.000008
15	Arginine (Arg)	0.25-25	0.00080	-0.00020
16	Asparagine (Asn)	0.25-25	0.01730	-0.00420
17	Lysine (Lys)	0.25-25	0.00680	-0.00320
18	Glutamine (Gln)	0.25-25	0.00170	-0.00090
19	Histidine (His)	0.25-25	0.00002	-0.000008
20	Tyrosine (Tyr)	0.25-25	0.00800	-0.00310
21	Tryptophan (Trp)	0.25-25	0.00130	-0.00030

LR: Linear range; b: Slope; a: Intercept.

The typical FAA profile of a conventional cooked ham analysed 12 days after production presented twenty one amino acids as shown in Figure 11.

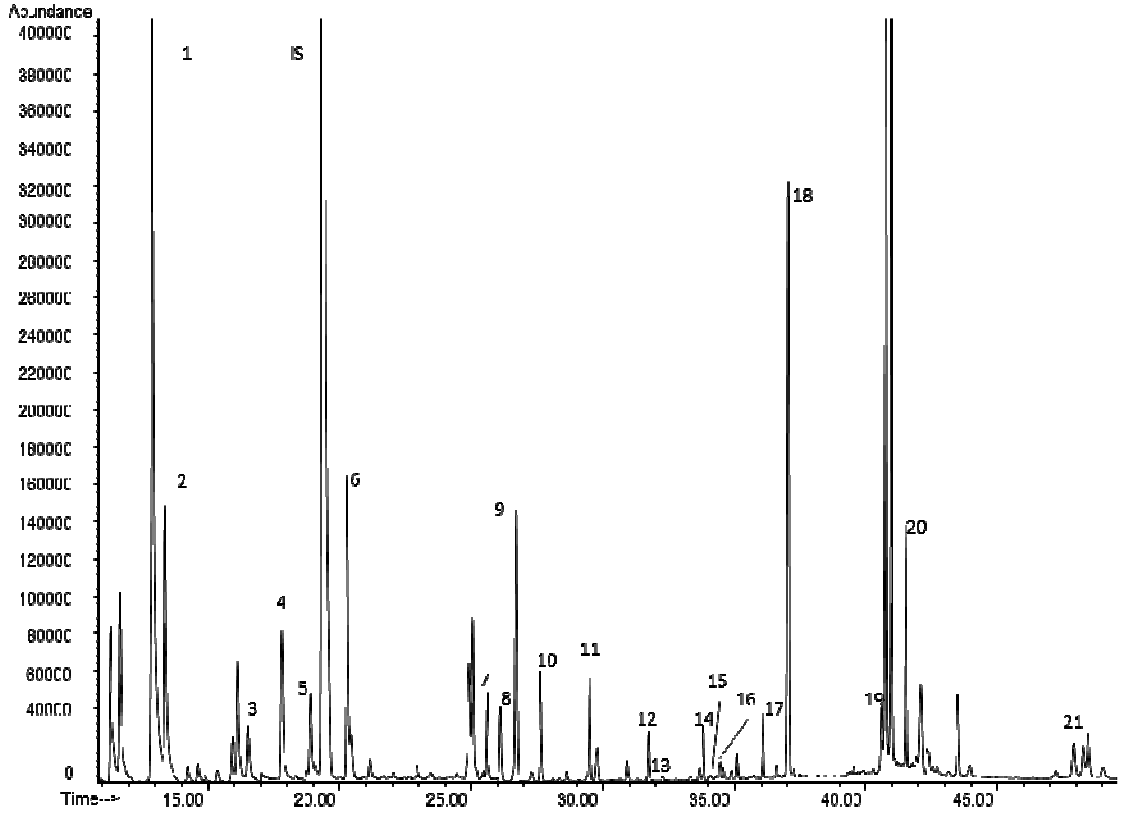

Figure 11: GC–MS separation of FAA in TIC mode after derivatization (see Table 5 for the peaks legend).

Table 6 shows FAA found in conventional cooked hams, the respective ions used for quantification, retention times and concentrations in cooked ham. Also, it is possible to notice that aliphatic amino acids (Ala, Gly, Val, Leu, Ile) are the main group arising almost 51% of total FAA concentration per 100 gram of dry matter of cooked ham. On the other hand, Arg and His were not detected and some FAA such as Met, Cys, Asp and Tyr were detected but not quantified being lower than LOQ.

Ile, Glu, Gln and Trp were found to be the major FAA in conventional cooked ham. Isoleucine (126 ± 0.22 mg/100 g dry matter) was the major FAA and compared with other meat products, its concentration was much higher than the content found in Iberian dry-cured ham (Jiménez-Martín, Ruiz et al. 2012, Lorenzo and Purriños 2013) and dry-cured fermented sausages (Roseiro, Gomes et al. 2010) 70 and 101 mg/100 g of dry matter and 47 mg/100 g of dry matter, respectively. Moreover, Glu presented lower concentration than the one observed in the meat products described before. Gln and Trp presented similar

behaviour than Ile, the contents obtained in conventional cooked hams were higher than Gln and Trp in sausages, pork, chicken and Iberian dry-cured products mentioned before.

Table 6: Free amino acid content (mg/ 100 g sample dry matter) in conventional cooked ham[a]

Amino acid	Ions	T_R (min)	Concentration
Alanine (Ala)	158	12.9	10.2 ± 0.90
Glycine (Gly)	218	13.4	3.48 ± 0.37
Valine (Val)	260	16.5	3.08 ± 0.04
Leucine (Leu)	200	17.8	2.54 ± 0.28
Isoleucine (Ile)	200	18.8	126 ± 0.22
Proline (Pro)	184	20.3	3.06 ± 0.88
Phenylalanine (Phe)	302	25.6	10.9 ± 3.89
Methionine (Met)	218	26.1	LOQ
Serine (Ser)	362	26.7	3.60 ± 0.11
Threonine (Thr)	404	28.0	2.02 ± 0.06
Aspartic acid (Asp)	302	29.5	LOQ
Hydroxyproline (Hyp)	388	31.7	2.76 ± 0.01
Cysteine (Cys)	406	32.3	LOQ
Glutamic acid (Glu)	416	33.7	36.6 ± 7.66
Arginine (Arg)	286	34.1	LOD
Asparagine (Asn)	417	34.4	1.40 ± 0.05
Lysine (Lys)	300	36.1	5.44 ± 0.15
Glutamine (Gln)	329	37.1	28.1 ± 4.57
Histidine (His)	196	40.6	LOD
Tyrosine (Tyr)	466, 302	41.6	LOQ
Tryptophan (Trp)	244	48.3	19.3 ± 1.21
Total FAA			257 ± 3.41

[a] Concentration in mg/100 g of dry matter, value are expressed in mean ± standard deviation;. Samples n=4. LOD= 0.32mg/100 g (dw). LOQ= 1mg/100 g (dw)

Jiménez-Martín, Ruiz et al. (2012) had determined the FAA profile and concentration in different meat products including lean pork, chicken stock and Iberian dry-cured ham and other food products. FAA content of this meat products were observed by the author that present a logical variation between products with Iberian dry-cured ham as the meat product with major quantity of total FAA (1125 mg/100 g of dry matter). It was explained by Sentandreu, Stoeva et al. (2003) that in this kind of products, meat suffer proteolysis during ripening periods. Although, the profile in all meat products described previously varied between them, one FAA, Glutamic acid, was the major FAA in all these meat products. Other FAA with relative importance in total FAA content were Cys and Gln for

lean pork, cysteine and Gln for chicken stock and for Iberian dry-cured ham Arg, Lys and Leu.

Conventional cooked ham presented clearly higher FAA content than lean pork due to the effect of curing salts and proteolysis that occurred during cooked ham production. However, in comparison with the other meat products described before, lower contents of FAA were observed. Dry-cured hams, such as Iberian dry-cured ham presented continuous proteolysis by muscle enzymes. The FAA content of cooked ham also differed from other meat products that were submitted to a curing step using nitrite and chloride salts (Nodake, Numata et al. 2013). Ala, Gly and Glu were the major FAA in cooked cured sausages and the total FAA was lower than that observed in cooked ham samples.

Toldrá, Mora et al. (2010) and Nodake, Numata et al. (2013) described that amino acids are responsible for taste and aroma formation by Strecker and Maillard reactions and that FAA can be released during resting time, brine injection, tumbling or massaging and cooking (Toldrá, Mora et al. 2010). Free amino acids like His, Val, Leu, Ile, Trp and Phe could be responsible for bitter taste and Ala, Ser, Gly, Thr and Pro for sweetness (Chaves-López, Paparella et al. 2011) and Glut is one of the FAA that contributes to umami taste in meat products (Nodake, Numata et al. 2013).

9.1.5. Volatile compounds profile

The volatile compounds found in conventional cooked ham analysed 12 days after production, are presented in Table 7. Twenty-one volatile compounds were identified including aldehydes (5), aromatic hydrocarbons (4), carboxylic acids (5), esters (3), terpens (2), sulphur compounds (2) and as internal standard (IS) L-Linalool. The aroma of cooked ham is a mixture of several volatile compounds. A unique compound responsible for the aroma of this product was not found, however, the volatile profile analysis helps to understand the characteristic aroma of cooked ham (Toldrá, Mora et al. 2010). Typical chromatographic profile of volatile compounds of cooked ham is shown in Figure 12.

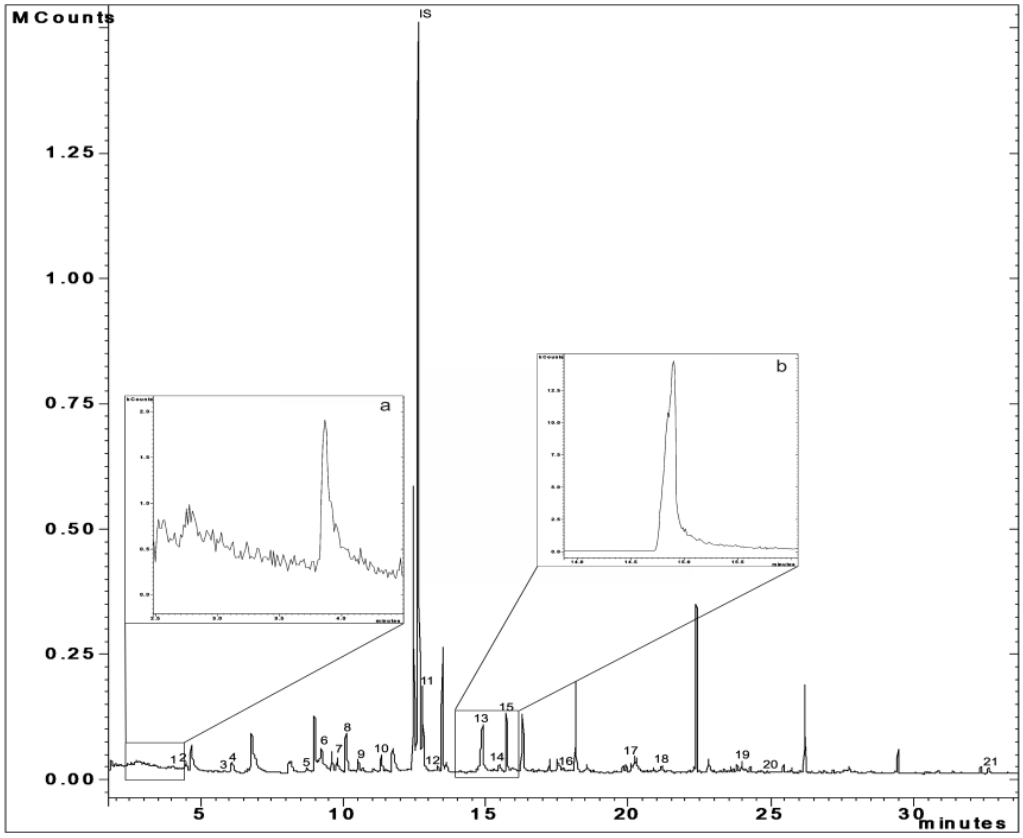

Figure 12: Volatile profile of conventional cooked ham in TIC mode added with IS (see Table 7 for the legend of peaks). *a*: Toluene peak in SIM mode (ion 91+65); *b*: Octanoic acid in SIM mode (ion 60)

The main chemical family is from carboxylic acids with 39% of total area followed by aldehydes that represent 32.1% of total area. Aldehydes and carboxylic acids can be produced from lipid oxidation (thermal degradation and/or chemical auto-oxidation), sugar fermentation and amino acids catabolism during cooking step and/or during storage (Comi and Iacumin 2012, Estévez, Morcuende et al. 2003). Major aldehyde was decanal whereas major carboxylic acid was octanoic acid. Benzaldehyde can be produced from phenylalanine by bacterial conversion. Nonanal and decanal can be formed by the reaction between cysteine and sugars, instead of lipids oxidation. These compounds can be related with off-flavours responsible for ham rejection by consumers (Leroy, Vasilopoulos et al. 2009). The third major chemical family are aromatics that represent 12.5% of total peak area, followed by sulphur compounds (8.5% of total peak area). Minor chemical families are terpens and esters that represents around 4% each.

Aromatic compounds could be generated by amino acid degradation (Toluene and Ethylbenzene) (Gianelli, Salazar et al. 2012). p-Xylene can be considered a contaminant according to Olivares, Navarro et al. (2011).

Sulphur compounds (1-methylthio-1-propene (E); 1-methylthio-1-propene (Z)) are derived from sulphur-containing amino acids that were detected in FAA analysis below the quantification level. This compounds suffered decomposition during cooking step (Ma, Hamid et al. 2012).

Terpens such as limonene and terpineol could be generated from raw material or animal feedstuffs or from plants that animals used to eat (Comi and Iacumin 2012).

Table 7: Volatile compounds in conventional cooked hams with their respectively CAS number, time retention and quantification ion, relative percentage of area and ratio peak area/Internal standard

Peak number	Volatile compounds	CAS Nº	R_T(min)	Kovats Index/ID	Ions	RA%	Ratio pA/IS
	Aldehyde						
2	Hexanal	66-25-1	4.49	790/S	56	2.39%	0.0137
5	Benzaldehyde	100-52-7	8.72	947/S	105+77	1.97%	0.0132
7	Octanal	124-13-0	9.79	1001/S	69+84+110	1.25%	0.0074
11	Nonanal	124-19-6	12.78	1103/S	57+82+124	12.20%	0.0734
15	Decanal	112-31-2	15.74	1205/S	57+82+124	14.45%	0.0897
18	Dodecanal	112-54-9	21.19	1401/T	81	0.19%	0.0013
	Carboxylic acids						
6	Hexanoic acid	142-62-1	9.25	995/S	60	12.50%	0.0712
13	Octanoic acid	124-07-2	14.85	1177/S	60	20.60%	0.1169
16	Nonanoic acid	112-05-0	17.63	1273/S	60	2.78%	0.0138
17	Decanoic acid	334-48-5	20.12	1380/S	60	1.89%	0.0109
20	Dodecanoic acid	143-07-7	25.01	1501/S	60	0.64%	0.0035
	Ester						
12	Octanoic acid methyl ester	111-11-5	13.33	1112/S	87+158	0.81%	0.0041
19	Dodecanoic acid methyl ester	111-82-0	23.99	1500/S	87+214	1.45%	0.0089
21	Hexadecanoic acid methyl ester	112-39-0	32.6	1928/S	87+270	1.36%	0.0084
	Aromatic						
1	Toluene	108-88-3	3.86	767/S	91+65	1.44%	0.0084
3	Ethylbenzene	100-41-4	5.84	865/S	91+106+77	1.52%	0.0088
4	p-Hylene	106-42-3	6.08	883/S	91+106+77	9.57%	0.0554
	Terpens						
9	Limonene	138-86-3	10.52	1036/S	68+93	3.95%	0.0241
14	Terpineol	98-55-5	15.48	1193/S	93+121	0.51%	0.0023
	Sulphur						
8	1-methylthio-1-propene (E)		10.11		69+84+110	5.83 %	0.0295
10	1-methylthio-1-propene (Z)		11.32		69+84+110	2.70%	0.0162

R_T(min) Retention time in minutes; ID identification, S identified by comparison with standard, T tentatively identified by NIST 05; Ions: Simple ion for quantification; RA%: Relative peak area expressed as % of total area of volatile compounds; pA/IS: ratio between arbitrary units of peak area and internal standard peak area

Comparison with volatile profile of cooked ham obtained by other authors (Leroy, Vasilopoulos et al. 2009, Comi and Iacumin 2012) indicates the presence of compounds similar to those found in this work, although these authors also mention formation of alcohol compounds due to microbial activity, but refer that its influence in cooked ham flavour is insignificant, being only important when cooked ham is sliced, packaged and stored.

9.2. Characterization of conventional ham after shelf-life period

It is well know that foods suffer modifications during shelf life due to chemical and biochemical reactions causing modification of colour, texture and taste and therefore will establish the limits of the product acceptance. To understand modifications that occur during shelf life namely the changes of colour, texture, proteolysis (peptides formation and FAA), volatiles were evaluated after 90 days of storage at 4±2ºC.

Mean Moisture content in conventional cooked hams at the end of shelf life was around 73.8%. Comparison between Moisture at 12 days and at the end of shelf life by *t*-test indicates a significant decrease ($p<0.001$). Moreover, pH (6.39) did not suffer significant modification once until 90 days no significant microorganism action occurred, according to industry information

9.2.1. Colour and texture parameters

Colour and Texture parameters are shown in Table 8, moreover results obtained at 12 days are also included for better assessment. A *t*-test was performed for comparison between 12 and 90 days of storage, *F*-value and significance are listed for each parameter.

Table 8: Comparison between colour attributes and texture profile analysis parameters of conventional cooked ham (Mean ± SD) analysed after 12 days and 90 days of preparation

			t-test	
Colour and TPA	**12 days**	**90 days**	***F***	***p***
*L**	62.3 ± 3.03	63.3 ± 1.53	3.48	0.374
*a**	11.8 ± 1.60	12.4 ± 1.67	0.08	0.531
*b**	7.79 ± 0.30	7.66 ± 1.06	11.5	0.772
Hardness (N)	51.7 ± 8.29	56.5 ± 10.3	0.18	0.307
Springiness (mm)	1.35 ± 0.08	1.27 ± 0.02	3.57	0.046
Cohesiveness (-)	0.72 ± 0.05	0.92 ± 0.01	5.20	<0.001
Gumminess (N)	37.1 ± 4.76	50.7 ± 9.23	1.15	0.001
Chewiness (N.mm)	42.7 ± 12.0	63.1 ± 11.8	1.03	0.010

After 90 days colour did not suffer significant modifications. Concerning texture characteristics, no significant differences were found in Hardness, however, other TPA values such as Springiness, Cohesiveness, Gumminess and Chewiness suffered a significant increase at the end of shelf life.

In conclusion, small modifications occur in texture characteristics of conventional cooked hams during a period of almost 3 months, which is a reasonable shelf life for this kind of product.

9.2.2. Protein degradation and peptides formation

Proteolysis that occurred between 12 and 90 days on PSDB protein fraction is summarized in Figure 13. Polypeptides that eluted between R_T 5 and 15 min decreased, although not significantly at 90 days (p = 0.913), whereas, polypeptides that eluted between R_T 15 and 25 min increased significantly (p<0.001), from 51% to 63% of RA%. Proteins from R_T 25 to 33 min significantly decreased (p<0.001) in RA% from 36% to 28%.

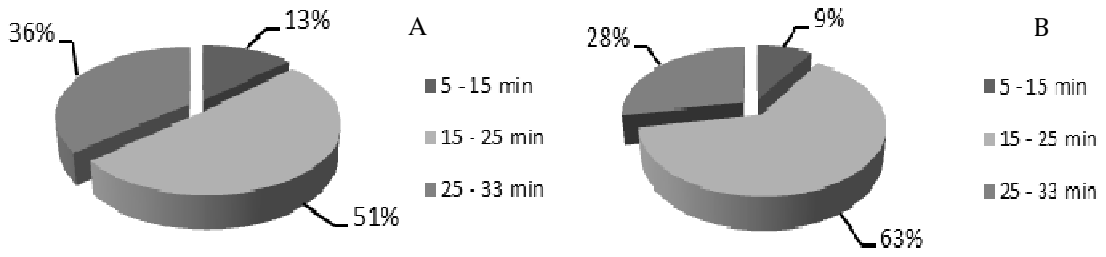

Figure 13: Protein degradation and peptides formation in PSDB protein fraction of conventional cooked hams analysed at (A) 12 days and (B) 90 days

Concerning PSSIB protein fraction a significant increase (p=0.001) was observed on RA% of R_T 15 to 25 min in comparison with the same peak at 12 days (76%). Protein from R_T 25 to 33min had a significantly decrease (p=0.033) in peak area and RA% too, from 37% to 24% at 90 days (Figure 14).

The modifications observed in peak area and RA% could be promoted by metabolism of psychotropic lactic acid bacteria LAB during storage (Leroy, Vasilopoulos et al. 2009, Comi and Iacumin 2012, Samelis, Kakouri et al. 2000), however, microbiological activity during storage is not feasible since no textural or colour and pH effects were highlighted in this work.

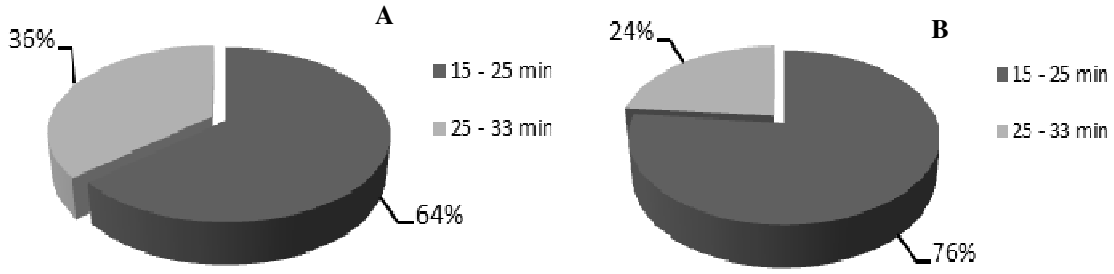

Figure 14: Protein degradation and peptides formation in PSSIB protein fraction of conventional cooked hams at 12 days (A) and 90 days (B).

9.2.3. Quantification of free amino acids

Changes in FAA concentration were observed in conventional hams after storage during 90 days (at the end of shelf life) in comparison with FAA content at 12 days. Table 9 shows a significant decrease in FAA concentrations of almost all amino acids, except Pro and Phe, where the decrease was not significant at 90 days, and also in the case of Gly, Val and His that presented an increase (although not significant p=0.735 for Gly) during storage time.

Most prominent reduction was found for Gln and Trp in relation with others FAA such as Ser, Glu, Leu, Lys, Thr, Asn and Ile. Hughes, Kerry et al. (2002) explained that this decrease could be due to degradation by Strecker reaction of FAA converting them into aldehydes, that are relevant flavour compounds or caused by the metabolism of amino acids by microorganism during storage (Ma, Hamid et al. 2012). In relation to umami flavour (due to presence of glutamate) it was observed that Glu reduced significantly but its proportion in relation with total FAA was similar from the beginning to the end of

storage ranging from 14.2% to 12.5% suggesting that the umami taste did not suffered a relevant modification.

Ile, Gln and Trp were the FAA with major decrease in concentration during shelf life period in conventional cooked ham. The decrease in some FAA could be associated with the formation of volatile compounds or biogenic amines during storage (Rabie, Peres et al. 2014). Some basic and aliphatic amino acids groups were found to increase during shelf-life in comparison with same group at 12 days.

Table 9: Comparison of FAA concentration in cooked ham analysed at 12 and 90 days (mg/100g of dry matter)

		Concentration		*t*-test	
Amino acid	**Ions**	**12 days**	**90 days**	***F*-value**	***p***
Alanine (Ala)	158	10.2 ± 0.90	9.31 ± 0.02	91.1	0.095
Glycine (Gly)	218	3.48 ± 0.37	3.54 ± 0.02	84.2	0.735
Valine (Val)	260	3.07 ± 0.04	3.30 ± 0.02	14.1	<0.001
Leucine (Leu)	200	2.54 ± 0.28	1.92 ± 0.02	80.1	0.008
Isoleucine (Ile)	200	126 ± 0.22	105 ± 1.65	70.9	<0.001
Proline (Pro)	184	3.06 ± 0.88	2.76 ± 0.01	91.1	0.495
Phenylalanine (Phe)	302	10.8 ± 3.88	10.7 ± 0.05	93.8	0.911
Methionine (Met)	218	LOQ	LOQ		
Serine (Ser)	362	3.60 ± 0.11	2.34 ± 0.02	56.4	<0.001
Threonine (Thr)	404	2.02 ± 0.06	1.56 ± 0.02	30.5	<0.001
Aspartic acid (Asp)	302	LOQ	LOQ		
Hydroxyproline (Hyp)	388	2.76 ± 0.01	2.40 ± 0.01	1.42	<0.001
Cysteine (Cys)	406	LOQ	LOQ		
Glutamic acid (Glu)	416	36.6 ± 7.66	25.6 ± 0.28	89.2	0.033
Arginine (Arg)	286	LOQ	LOQ		
Asparagine (Asn)	417	1.40 ± 0.05	1.13 ± 0.02	19.8	<0.001
Lysine (Lys)	300	5.44 ± 0.15	4.14 ± 0.11	3.33	<0.001
Glutamine (Gln)	329	28.1 ± 4.57	6.94 ± 0.18	88.4	<0.001
Histidine (His)	196	LOD	20.7 ± 0.01	96.0	<0.001
Tyrosine (Tyr)	466, 302	LOQ	LOQ		
Tryptophan (Trp)	244	19.3 ± 1.21	2.39 ± 0.22	92.4	<0.001
Total FAA		257 ± 3.41	204 ± 2.27	7.41	<0.001

Value are expressed in Mean ± standard deviation; Concentration in mg/100g of dry matter. LOD= 0.32mg/100g (dw). LOQ= 1mg/100g (dw)

The content of FAA during storage was studied in products with different processing conditions such as ripening in dry-cured products (Nodake, Numata et al. 2013, Jurado, García et al. 2007, Hughes, Kerry et al. 2002, Yamanaka, Akimoto et al. 2005) and an

increase during storage period was observed. This effect was caused by the activity of enzymes and microorganisms that occur during ripening of those products, however, in cooked ham these enzymes are not active due to the thermal process used in their production. However, in cooked ham this effect was not expected due to the thermal processing step during manufacture of cooked ham.

9.2.4. Volatile compounds

The volatile profile during shelf life did not suffered qualitative modification, since generation of new volatile compounds was not observed. However, quantitative differences occur, as shown in Table 10. CAS number, R_T (min), Kovats index and quantification ions from volatile compounds identified in conventional ham were previously described in Table 7.

Aldehydes were the main group of volatile compounds with 36.2 % of total area. All aldehydes increased significantly at the end of shelf life due to the autoxidation of lipids. Total carboxylic acids decreased in RA% in comparison with 12 days representing 25.1% of total area. Octanoic, decanoic and dodecanoic acids increased while hexanoic and n-nonanoic acids decreased in relation with peak area at 12 days. Total esters did not suffered changes maintaining approximately 4% of total area. Sulphur compounds did not suffered significant modifications. Terpens and aromatics compounds, except p-Xylene increased. Aromatic compounds increased due to their relation with proteolysis and amino acids degradation (Gianelli, Salazar et al. 2012). Terpens probably come from animal feeding and ingredients like black pepper (Gardini, Tabanelli et al. 2013) and some authors (Holm, Adamsen et al. 2013) found a decrease of these compounds in meat products during storage, while other authors (Huang, Xiong et al. 2013, Gardini, Tabanelli et al. 2013) found an increase of terpens which is difficult to explain in cooked ham.

t-test was performed between the ratio peak area / internal standard peak area at 12 and 90 days. A significant increase of this ratio at 90 days was observed for all compounds in comparison with the results obtained at 12 days. The exceptions were hexanoic acid, n-nonanoic acid, p-xylene and sulphur compounds that did not suffered significant changes.

Table 10: Volatile compounds in conventional cooked hams analysed at 12 and 90 day after production expressed as relative % of area and ratio between peak area /internal standard peak area and *F*-values of *t*-test performed with pA/IS

	Peak area				*t*-test	
	12 days		90 days		*F*- value	*p*
Volatile compounds	RA%	pA/IS	RA%	pA/IS		
Aldehyde						
Hexanal	2.39%	0.0137	2.08%	0.0302	0.242	<0.001
Benzaldehyde	1.97%	0.0132	2.12%	0.0293	0.022	0.022
Octanal	1.25%	0.0074	3.05%	0.0427	0.489	<0.001
Nonanal	12.20%	0.0734	24.58%	0.3559	2.827	<0.001
Decanal	14.45%	0.0897	3.22%	0.0455	10.052	0.038
Dodecanal	0.19%	0.0013	1.15%	0.0156	0.573	<0.001
Carboxylic acids						
Hexanoic acid	12.50%	0.0712	5.46%	0.0791	0.808	0.160
Octanoic acid	20.60%	0.1169	15.63%	0.2156	0.065	<0.001
nNonanoic acid	2.78%	0.0138	0.79%	0.0116	38.521	0.678
Decanoic acid	1.89%	0.0109	1.63%	0.0231	0.022	<0.001
Dodecanoic acid	0.64%	0.0035	1.56%	0.0221	0.293	<0.001
Ester						
Octanoic acid methylester	0.81%	0.0041	1.10%	0.0168	2.250	<0.001
Dodecanoic acid methylester	1.45%	0.0089	2.52%	0.0418	5.315	0.001
Hexadecanoic acid methylester	1.36%	0.0084	1.18%	0.0166	214.597	0.015
Aromatic						
Toluene	1.44%	0.0084	1.46%	0.0209	0.329	<0.001
Ethylbenzene	1.52%	0.0088	11.61%	0.1653	6.497	<0.001
p-Xylene	9.57%	0.0554	4.60%	0.0653	30.645	0.138
Terpens						
Limonene	3.95%	0.0241	9.11%	0.1350	3.029	<0.001
Terpineol	0.51%	0.0023	2.67%	0.0415	5.485	0.001
Sulphour compound						
1-methylthio-1-propene (E)	5.83 %	0.0295	3.30%	0.0464	273.589	0.079
1-methylthio-1-propene (Z)	2.70%	0.0162	1.18%	0.0165	6.217	0.877

RA%: Relative peak area expressed as % of total area of volatile compounds; pA/IS: ratio between arbitrary units of peak area and internal standard peak area

9.3. Characteristics of conventional cooked ham

The conventional cooked hams produced for this study presented similar quality characteristics than cooked hams described in bibliography, denoting that this type of food presents a high moisture and protein content. Although, the fat content of cooked ham is highly variable, the conventional cooked ham studied presented low fat content. As far as Na^+ and K^+ content are concerned, it should be highlighted that it contains similar levels of

K^+ when compared with the description of Portuguese table of food composition (INSA 2006) and half of the content described for Na^+.

Regarding colour and texture, which are important attributes of conventional cooked ham, these parameters were similar to those described in bibliography, although as it is well known, sensory and final attributes of meat products will oscillate depending on raw materials used and technological process applied for production (Desmond, Kenny et al. 2000). These parameters are affected by intrinsically and extrinsically characteristics principally such as extent of heating, moisture content, degree of myofibrillar proteins denaturation and proteolysis, the content of connective tissue and fat as described by Toldrá, Mora et al. (2010). In addition this author refers that the content of intramuscular fat also gives a positive influence on some texture and appearance traits.

Concerning protein and peptides profile of PSDB and PSSIB protein fractions, of conventional cooked ham presented significant modifications of native proteins and extensive proteolysis due to the effects of salts, phosphates and temperature that promotes protein denaturation and peptides and polypeptides formation compared with typical chromatographic profile of raw pork meat.

This product, due to its elevated tumbling time, denote a high content of FAA that decrease at the end of shelf-life, as result of being converted into volatile compounds by chemical reactions (Maillard, Strecker). Also, conventional cooked ham shown volatile compounds mostly generated from lipids thermal degradation and/or chemical auto-oxidation (aldehydes and acids). At the end of shelf life period relative areas of aldehydes increased. This aspect is of major relevance because if it occurs in great extension can cause rejection of cooked ham by consumers because volatiles compounds originating from lipid oxidation are related with acceptability of meat products by consumers (Leroy, Vasilopoulos et al. 2009). However it should be highlighted that during shelf-life period the product did not suffer significant modifications in texture and colour attributes, which are important parameters to maintain stability of the final product and the preference of consumer.

The major characteristics in conventional cooked ham production process are the high tumbling time applied to meat cuts to improve diffusion of brine and to solubilize brine salts. According to Li, Szczepaniak et al. (2011) tumbling time is very important to guarantee the best final quality characteristics of cooked ham. Also this author found that the major diffusion and solubilisation of proteins is between 4 and 6 h. These authors concluded that in cooked ham process 4h would be practical to apply in cooked ham

production maintaining both quality and sensory characteristics. A decrease in tumbling time applied to the production of this conventional cooked ham while maintaining sensory and quality characteristics is a challenge.

Section B : Cooked ham with reduced tumbling time: effects of spent *Saccharomyces* yeast extract addition and cooking time

10. Effects of addition of spent yeast extract on the production of cooked ham with reduced tumbling time and influence of cooking time

10.1. Characterization of spent yeast extract

The total protein content of yeast extract was 10.4±0.02 mg ml^{-1} and the pH was 5. Spent yeast extract presented proteolytic activity, the value obtained according to Cupp-Enyard (2008) method was 0.34 U ml-1.

Protein chromatographic profile of spent yeast extract stored at 4ºC and after 1 hour incubation at 70ºC is presented in Figure 15. The main peak area in spent yeast extract stored at 4°C (Figure 15 A) has retention time (R_T) 25-33 min (66.3%) corresponding to proteins with molecular weight >60kDa. R_T 15-25 min (25.4%) is the second main peak area corresponding to proteins and polypeptides >14 kDa and < 60 kDa and R_T 5-15 min represented only the 8.3% of total peak area and belongs to polypeptides with molecular weight <14 kDa. In Figure 15B it was observed that the peak area between R_T 25min to 33 min was completely degraded by heat effect and an increase in relative peak area of RT 15-25 min (68.2%) and R_T 5-15 min (31.8%) was notably seen.

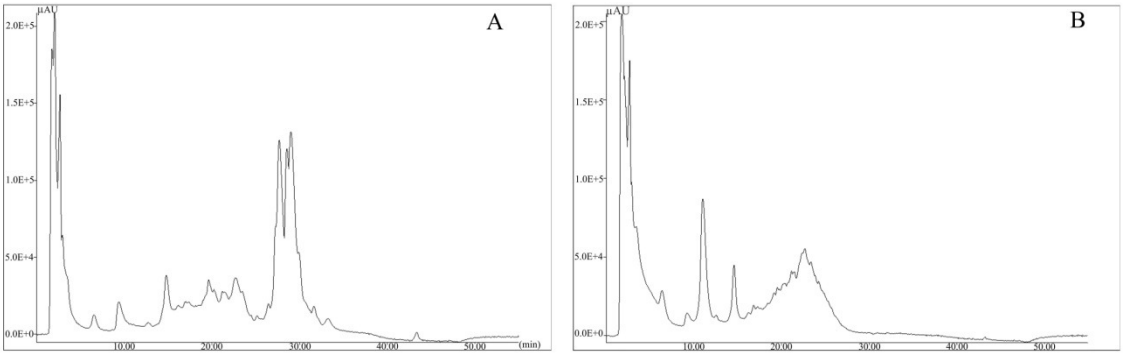

Figure 15: Chromatographic profile of spent yeast extract. A: stored at 4°C; B: incubated 1 hour at 70°C

These chromatograms highlight that proteins from spent yeast extracts suffer proteolysis when submitted to heat treatment. The spent yeast extract that will be used as ingredient in the production of cooked ham contains proteins and enzymes that are hydrolysed at temperatures usually applied in cooked ham production. Thus, the enzymatic activity is lost after ham cooking.

10.2. Characterization of cooked ham with and without spent yeast extract at different cooking times

10.2.1. Effect of addition of spent yeast extract and cooking time in the proximal composition

Proximal composition and pH of control cooked hams added of 1% acetate buffer and cooked hams added 1% spent yeast extract at different cooking times are presented in Table 11. Proximal composition of cooked hams added of 1% acetate buffer indicates that moisture ranged from 73.4 to 76.4%. Protein, fat and ash percentages varied from 14.1 to 15.40%; 2.56 to 4.60% and 3.38 to 3.88%, respectively. Mean pH value of these cooked ham varied from 6.16 and 6.23 which is similar to those reported by Tomović, et al. (2013) and Li, et al. (2011) that mentioned pH values of 6.25 and 6.2, respectively. The moisture content is in agreement with other works from literature that studied cooked ham composition (Moretti, et al., 2009; Tomović, et al., 2013). Similar values were also observed for total protein (Zell, Lyng, Morgan, & Cronin, 2012), fat (Válková, Saláková, Buchtová, & Tremlová, 2007) and ash content (Casiraghi, et al., 2007). With regard to cooked hams added with spent yeast extract, similar contents were observed except on protein and ash contents. Protein content of those samples ranged between 16.2 to 17.0% and ash content varied between 3.65 to 3.93%. These increases are due to the protein content and intercellular composition of spent yeast extracts.

Table 11: Proximal composition of cooked hams added with 1% acetate buffer and 1% spent yeast extract at different cooking times

	Cooked ham added with 1% acetate buffer				Cooked ham added with 1% spent yeast extract			
	1.5 h	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
pH	6.20 ± 0.24	6.23 ± 0.13	6.16 ± 0.18	6.22 ± 0.18	6.27 ± 0.12	6.14 ± 0.24	6.26 ± 0.16	6.23 ± 0.12
Moisture	75.4 ± 1.33	73.4 ± 3.57	75.8 ± 0.37	76.4 ± 0.44	74.3 ± 2.13	74.6 ± 0.49	74.9 ± 0.70	74.9 ± 0.45
Protein	14.4 ± 0.47	14.7 ± 0.18	15.4 ± 0.19	14.1 ± 0.36	16.3 ± 0.80	17.0 ± 0.64	16.2 ± 0.93	16.3 ± 0.84
Lipids	4.60 ± 2.72	4.04 ± 2.85	2.56 ± 0.78	2.57 ± 0.80	3.90 ± 1.70	3.03 ± 0.59	3.29 ± 1.07	3.48 ± 0.27
Ash	3.60 ± 0.16	3.38 ± 0.18	3.48 ± 0.26	3.88 ± 0.32	3.93 ± 0.34	3.91 ± 0.33	3.91 ± 0.18	3.65 ± 0.42
Na+	0.92 ± 0.10	0.89 ± 0.07	0.87 ± 0.10	0.80 ± 0.00	0.73 ± 0.06	0.90 ± 0.00	0.80 ± 0.00	0.96 ± 0.12
K+	0.32 ± 0.03	0.36 ± 0.05	0.37 ± 0.05	0.33 ± 0.04	0.35 ± 0.02	0.35 ± 0.05	0.36 ± 0.03	0.32 ± 0.03

Results presented in %w/w (Mean ±SD). Samples n=4 for each cooking time

Two-way ANOVA (Table 12) was performed to assess the effect of yeast addition and cooking time on proximal composition of cooked ham. A significant interaction between yeast addition and cooking times was observed for moisture, protein and ash being the last

two components the most influenced because of the higher F-value in comparison with moisture. Two-way ANOVA outcomes revealed significant effects of the yeast addition on protein (F=208.739; p=0.000) and ash (F=20.402; p=0.000) and significant effects of cooking time for moisture (F=4.548, p=0.005), protein (F=5.828; p=0.001), lipids (F=2.952, p=0.037); and K+ (F=3.338; p=0.029).

Table 12: Two-way ANOVA results of yeast addition and cooking time effects in proximal composition and pH of cooked hams added with 1% acetate buffer and cooked hams 1% spent yeast extract analysed at 12 days

	Effects of yeast addition		**Effects of cooking time**		**Interaction**	
	F	***p***	***F***	***p***	***F***	***p***
pH	1.205	0.275	0.221	0.881	1.951	0.127
Moisture	2.996	0.087	4.548	0.005	3.538	0.018
Protein	208.739	0.000	5.828	0.001	8.064	0.000
Lipids	0.023	0.879	2.952	0.037	2.545	0.061
Ash	20.402	0.000	0.888	0.451	9.536	0.000
Na^+	2.722	0.109	2.738	0.061	5.116	0.006
K^+	0.030	0.864	3.338	0.029	0.892	0.454

The effect of spent yeast extract addition in cooked ham production influenced considerably the protein and ash content, since the respective F-values were higher than those of the effect of cooking time for the same parameters. This effect results from the protein content and intercellular composition of spent yeast extracts.

10.2.2. Influence of addition of spent yeast extract and cooking time in CIE Lab and TPA analysis

CIE Lab and TPA attributes of cooked ham added with 1% acetate buffer and cooked ham added 1% spent yeast extract are shown in Table 13. Prominent differences were observed in CIE L^* value and hardness that were higher in cooked ham added of 1% spent yeast extract than in cooked ham added with 1% acetate buffer. Two-way ANOVA (Table 14) was performed to assess the effect of yeast addition and cooking time on CIE Lab and TPA attributes.

Table 13: CIE Lab and TPA attributes of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract at different cooking times after 12 days of production

	Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
Attributes	**1.5 h**	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
*L**	60.8 ± 0.67	62.2 ± 2.23	60.2 ± 0.35	61.0 ± 0.92	65.4 ± 2.81	62.8 ± 1.93	63.1 ± 2.50	62.7 ± 5.24
*a**	12.0 ± 1.18	11.7 ± 1.33	12.5 ± 2.75	12.4 ± 1.61	10.3 ± 1.76	12.1 ± 1.42	11.8 ± 1.38	11.7 ± 3.21
*b**	8.83 ± 0.65	8.45 ± 0.88	8.67 ± 0.68	9.12 ± 0.49	7.94 ± 0.85	7.92 ± 0.71	8.05 ± 0.56	8.55 ± 0.97
Hardness (N)	44.1 ± 12.7	49.2 ± 9.83	51.4 ± 7.90	44.2 ± 9.18	57.3 ± 9.37	56.6 ± 14.1	58.2 ± 12.1	50.8 ± 4.85
Springiness (mm)	1.30 ± 0.10	1.32 ± 0.07	1.25 ± 0.10	1.33 ± 0.12	1.35 ± 0.10	1.36 ± 0.06	1.38 ± 0.08	1.30 ± 0.03
Cohesiveness (-)	0.73 ± 0.05	0.70 ± 0.04	0.64 ± 0.06	0.72 ± 0.06	0.72 ± 0.06	0.67 ± 0.04	0.71 ± 0.07	0.71 ± 0.03
Gumminess (N)	33.1 ± 9.08	36.3 ± 8.82	32.5 ± 5.16	32.2 ± 8.23	41.1 ± 7.88	37.6 ± 9.70	41.4 ± 9.19	36.2 ± 5.42
Chewiness (Nmm)	40.8 ± 15.7	46.0 ± 10.8	40.9 ± 7.30	43.2 ±13.6	55.7 ± 12.3	47.2 ± 11.6	57.2 ± 13.6	43.6 ± 10.0

Results are presented as Mean ± SD. Samples n=4 for each cooking time.

Two-way ANOVA outcomes are summarized in Table 14, significant effects of yeast addition were observed on *L** (F=18.93; p=0.000), *b** (F=18.24; p=0.000), hardness (F=14.45; p=0.000), gumminess (F=10.68; p=0.002) and chewiness (F=12.59; p=0.001) and significant effects of cooking time for *b** (F=3.19; p=0.028), cohesiveness (F=4,71; p=0.004) and chewiness (F=7.67; p=0.000).

Table 14: Two-way ANOVA results of yeast addition and cooking time effects in CIE Lab and TPA analysis of cooked ham added with 1% acetate buffer and 1% spent yeast extract at different cooking times stored 12 days

	Effects of yeast addition		Effects of cooking time		Interaction	
	F	***p***	***F***	***p***	***F***	***p***
*L**	18.93	0.000	1.44	0.238	2.32	0.081
*a**	2.73	0.102	1.27	0.291	1.10	0.354
*b**	18.24	0.000	3.19	0.028	0.29	0.836
Hardness (N)	14.45	0.000	1.92	0.132	0.33	0.804
Springiness (mm)	1.39	0.242	0.79	0.502	5.92	0.001
Cohesiveness (-)	0.11	0.736	4.71	0.004	4.61	0.005
Gumminess (N)	10.68	0.002	0.67	0.575	1.11	0.350
Chewiness (Nmm)	12.59	0.001	7.67	0.000	4.12	0.009

The higher F values observed in effects of yeast addition indicate that its effect is more important than the effect of cooking time conditions. Hardness was the TPA parameter with a prominent effect between cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract. No studies were found concerning the addition of yeast extracts to produce cooked ham, although the addition of other ingredients to meat

products was performed by other authors. Campagnol, dos Santos et al. (2011) did not find any difference between fermented sausages produced without addition or with addition of *saccharomyces* yeast. Other authors tried addition of pectin or whey proteins to increase hardness while reducing fat intake. Cardoso, Henry et al. (2012) found that the addition of pectin 1.5% (w/w) modified the texture of cooked ham, while Dutra, Cardoso et al. (2012) found no significant differences (p>0.05) in textural properties of low-fat cooked ham produced with the addition of whey proteins in comparison with cooked ham without addition of whey proteins. The addition of spent yeast extract presented similar effect on texture as soluble pectin, and therefore can be used to stabilise the structure of this type of meat product.

10.2.3. Proteolysis and peptides formation

Typical chromatograms obtained for PSDB protein fraction of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract are presented in Fig. 16 A and B, respectively. Main peak areas in cooked hams were R_T15 to 25 min. The peak areas of R_T 25 to 33 min in cooked ham added with 1% acetate buffer was higher when compared with that of cooked ham added with 1% spent yeast extract, indication higher amount of proteins with molecular weight higher than 60 kDa.

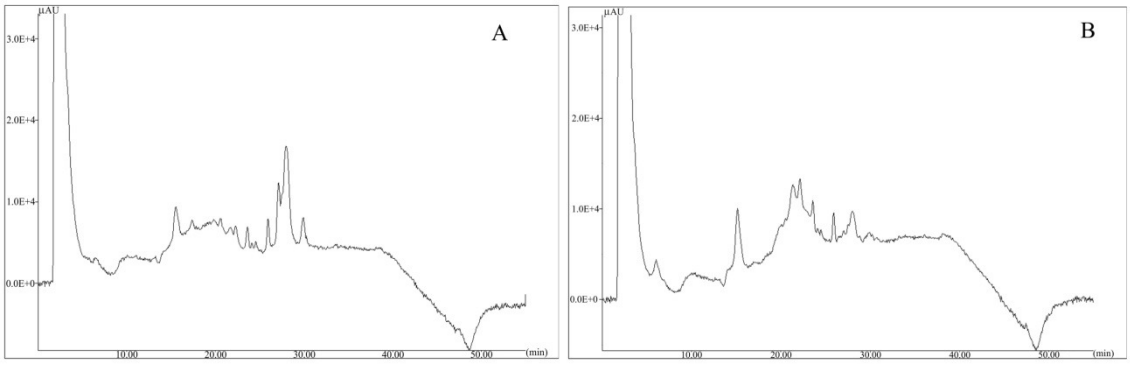

Figure 16: PSDB protein fraction of cooked ham at 1.5 h of cooking time: A) PSDB protein fraction of cooked ham added with 1% acetate buffer and B: PSDB protein fraction of cooked ham added with 1% spent yeast extract

Concerning chromatograms of PSSIB protein fraction (Figure 17) R_T 15 to 25 min was the main peak area in cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract (Fig. 17 A and B).

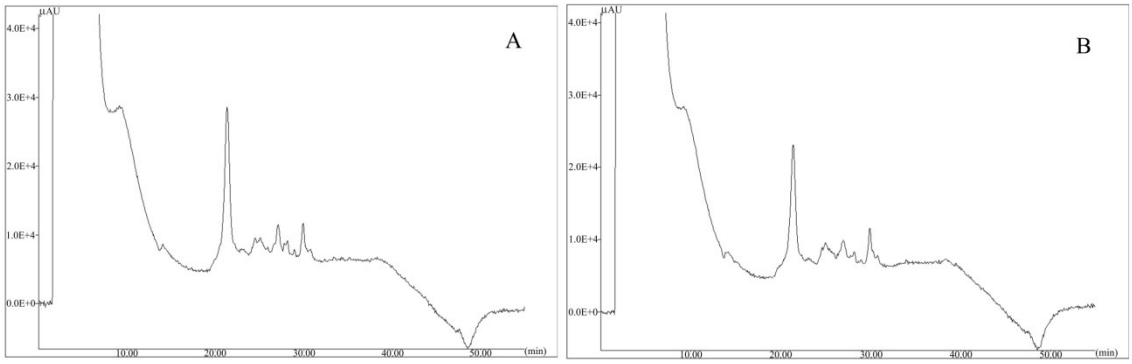

Figure 17: Chromatographic profile of PSSIB protein fraction of cooked hams at 1.5h of cooking time: A) cooked ham added with acetate buffer and B) cooked ham added with spent yeast extract

Relative peak areas of the two protein fractions (PSDB and PSSIB) at different cooking times expressed as % of total peak area are presented in Table 15. Differences between peak areas of cooked ham with 1% acetate buffer and cooked ham added with 1% spent yeast were observed.

Table 15: Relative peak area expressed as percentage of PSDB or PSSIB protein fractions of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract at different cooking times stored 12 days

	Cooked ham added with acetate buffer				**Cooked ham added with spent yeast extract**			
	1.5 h	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
PSDB*								
R_T 5-15 min	5.4	5.2	7.1	6.2	13.4	13.2	12.3	10.3
R_T 15-25 min	58.3	62.5	76.6	70.1	68.1	74.3	74.8	76.7
R_T 25-33 min	36.3	32.3	16.3	23.7	18.5	12.5	12.9	13.0
PSSIB**								
R_T 5-15 min	----	----	----	----	----	----	----	----
R_T 15-25 min	68.1	61.7	61.8	56.4	49.8	68.5	60.7	70.2
R_T 25-33 min	31.9	38.3	38.2	43.6	50.2	31.3	39.3	29.8

*Results are presented as relative % of total peak area of PSDB protein fraction; ** Results are presented as relative % of total peak area of PSSIB protein fraction; Samples n=4 for each cooking time

Two-way ANOVA outcomes are summarized in Table 16, significant effects of yeast addition were observed on peak area from polypeptides with molecular weight <14 kDa

(R_T 5-15 min) (F=60.171; p=0.000) and proteins with molecular weight >60 kDa (R_T 25-33 min) (F=15.387; p=0.001) from PSDB protein fraction. Since proteins from yeast extract after 1h heating at 70 ºC suffer hydrolysis that results in an increase of relative areas of proteins and polypeptides with molecular weight <14 kDa (R_T 5-15 min) and degradation of proteins with < 60kDa (R_T 15-25 min) these results point out that yeast proteins are extracted in the PSDB protein fraction of cooked ham. Yeast proteins contributed to increase relative areas of proteins and polypeptides with molecular weight <14 kDa (R_T 5-15 min) when compared with cooked ham added with acetate buffer, and consequently the relative area of proteins with T_R 25-33 min decreases. No significant effects of cooking time or interaction were found in PSDB protein fraction degradation (R_T 25-33 min) or on peptides formation (R_T 5-15 min and R_T 15-25 min). Concerning PSSIB protein fraction the effects yeast addition and cooking time were not significant, thus, the addition of yeast extract has not promoted an increase of proteolysis of PSSIB protein fraction.

Table 16: Two-way ANOVA results of yeast addition and cooking time effects on protein degradation and peptides formation of cooked ham added with 1% acetate buffer and 1% spent yeast extract stored 12 days

	Effects of yeast addition		**Effects of cooking time**		**Interaction**	
	F	***p***	***F***	***p***	***F***	***p***
PSDB						
R_T 5-15 min	60.171	0.000	0.547	0.655	1.499	0.240
R_T 15-25 min	3.313	0.081	2.362	0.096	0.679	0.573
R_T 25-33 min	15.387	0.001	2.792	0.062	1.272	0.306
PSSIB						
R_T 5-15 min	----	----	----	----	----	----
R_T 15-25 min	0.003	0.957	0.186	0.905	1.280	0.304
R_T 25-33 min	0.003	0.957	0.186	0.905	1.280	0.304

10.2.4. Quantification of free amino acids

Results obtained for FAA in cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extracts for each cooking time at 12 days of storage are presented in Table 17. Arg, Asp, Cys, His and Met were not quantified or detected in

cooked hams. Thr was under LOQ in cooked ham added with 1% acetate buffer and quantified in cooked ham added with 1% spent yeast extract.

Table 17: FAA content of cooked ham added with 1% acetate buffer and 1% spent yeast extract at different cooking times stored 12 days [a]

		Cooked ham added with 1% acetate buffer				Cooked ham added with 1% spent yeast extract			
FAA	**Ions**	**1.5 h**	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
Ala	158	6.65 ± 0.04	7.27 ± 0.91	8.42 ± 0.39	8.77 ± 0.88	9.30 ± 0.66	10.2 ± 0.35	11.4 ± 0.52	10.8 ± 0.50
Gly	218	2.13 ± 0.31	2.71 ± 0.33	2.77 ± 0.24	2.78 ± 0.35	3.08 ± 0.16	3.51 ± 0.29	3.69 ± 0.12	3.49 ± 0.09
Val	260	1.69 ± 0.26	2.46 ± 0.23	2.87 ± 0.37	1.82 ± 0.51	3.08 ± 0.25	2.97 ± 0.09	2.88 ± 0.03	2.77 ± 0.65
Leu	200	1.21 ± 0.02	1.57 ± 0.15	2.44 ± 0.59	1.98 ± 0.02	2.93 ± 0.48	2.36 ±0.09	2.42 ± 0.13	3.06 ± 0.78
Ile	200	124 ± 0.20	96.7 ± 1.38	116 ± 11.3	105 ± 5.27	101 ± 0.43	102 ± 0.35	101 ± 1.83	96.0 ± 5.00
Pro	184	2.18 ± 0.41	2.13 ± 0.10	2.02 ± 0.06	2.37 ± 0.62	2.31 ± 0.60	2.62 ± 0.61	3.49 ± 0.70	2.78 ± 0.64
Phe	302	8.84 ± 0.87	9.09 ± 2.12	10.1 ± 2.11	16.0 ± 0.71	8.79 ± 1.70	11.6 ± 1.57	16.7 ± 1.29	15.4 ± 3.50
Met	218	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Ser	362	1.47 ± 0.04	1.74 ± 0.19	2.84 ± 1.05	2.76 ± 0.00	3.85 ± 0.52	3.34 ± 0.44	3.90 ± 0.10	4.06 ± 0.94
Thr	404	LOQ	LOQ	LOQ	LOQ	2.20 ± 0.34	1.90 ± 0.07	2.46 ± 0.45	2.56 ± 0.58
Asp	302	LOQ	LOD	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Hyp	388	2.44 ± 0.02	2.19 ± 0.01	LOQ	LOQ	2.42 ± 0.02	2.44 ± 0.02	2.52 ± 0.01	2.49 ± 0.00
Cys	406	LOD	LOD	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Glu	416	39.4 ± 21.6	34.4 ± 23.6	54.5 ± 10.1	75.9 ± 4.16	42.9 ± 11.2	40.8 ± 12.2	34.1 ± 0.02	40.2 ± 20.8
Arg	286	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Asn	417	1.15 ± 0.02	1.10 ± 0.01	1.32 ± 0.04	1.39 ± 0.00	1.41 ± 0.11	1.22 ± 0.15	1.41 ± 0.08	1.56 ± 0.18
Lys	300	2.60 ± 0.23	3.25 ± 0.14	4.23 ± 0.79	2.98 ± 1.17	4.73 ± 0.20	4.43 ± 0.90	5.32 ± 0.25	4.87 ± 0.78
Gln	329	10.2 ± 7.30	7.98 ± 6.82	11.4 ± 1.03	4.63 ± 0.21	16.9 ± 6.53	12.0 ± 6.02	20.3 ± 3.51	13.8 ± 0.00
His	196	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Tyr	466, 302	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Trp	244	8.24 ± 0.00	1.12 ± 0.02	9.17 ± 0.00	3.37 ± 2.59	18.2 ± 0.00	20.9 ± 0.00	26.0 ± 2.23	19.8 ± 0.36
Total		205 ± 6.79	171 ± 34.3	235 ± 11.5	218 ± 13.4	213 ± 8.9	210 ± 17.3	220 ± 24.1	182 ± 33.9

[a] Value are expressed in Mean ± SD; Concentration in mg/100g of dry matter. Samples n=4 for each cooking time. LOD= 0.32mg/100g (dw). LOQ= 1mg/100g (dw)

Some FAA, namely, Ala, Gly, Val, Ser, Thr, Lys, Trp were higher in cooked ham added with spent yeast extract, probably owed to yeast FAA composition. Campagnol, dos Santos et al. (2011) explained that yeast extracts are characterized by a high content of amino acids. In turn, FAA profile observed in cooked ham added with 1% acetate buffer was Ile > Glu > Gln > Trp. Whereas, in cooked ham added with 1% spent yeast extract this profile presented a slight variation in Gln and Trp, being Ile > Glu > Trp > Gln. In both processing methods the same FAA were the most abundant FAA regardless the cooking time applied.

Additionally, in both processing methods, the main FAA group was the aliphatic group due to the inclusion of Ile followed by acids FAA group.

Two-way ANOVA was used to understand the effect of yeast addition and cooking time on FAA content in cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract and their interaction (Table 18).

Table 18: Two-way ANOVA results of yeast addition and cooking time effects on FAA of cooked ham added with 1% acetate buffer and 1% spent yeast extract at different cooking times stored 12 days

	Effects of yeast addition		**Effects of cooking time**		**Interaction**	
	F	***p***	***F***	***p***	***F***	***p***
Ala	159.949	0.000	18.839	0.000	1.141	0.349
Gly	109.112	0.000	12.000	0.000	0.460	0.713
Val	29.549	0.000	4.370	0.014	5.161	0.007
Leu	21.286	0.000	2.161	0.126	4.268	0.018
Ile	36.454	0.000	13.892	0.000	11.753	0.000
Pro	14.727	0.001	1.877	0.160	3.186	0.042
Phe	9.550	0.005	20.544	0.000	5.660	0.004
Ser	33.901	0.000	3.285	0.043	1.209	0.333
Thr	8.483	0.012	2.713	0.088	2.316	0.152
Hyp	1.750	0.227	898.115	0.000	895.417	0.000
Glu	1.583	0.222	0.871	0.472	1.355	0.284
Asn	8.347	0.010	5.295	0.009	0.452	0.719
Lys	43.799	0.000	4.323	0.014	1.205	0.329
Gln	4.678	0.043	0.847	0.484	0.473	0.705
Trp	112.003	0.000	12.672	0.000	1.771	0.189
Total FAA	0.014	0.907	4.418	0.013	4.549	0.012

Two-way ANOVA indicate that the addition of spent yeast extract to cooked ham has a significant effect in all quantified FAA except Hyp and Glu. The effect of cooking time was also significant in all quantified FAA except Leu, Pro, Thr, Glu and Gln.

10.2.5. Volatile compounds profile

Volatile compounds were determined in cooked ham added with 1% acetate buffer and in cooked ham added with 1% spent yeast extract expressed as relative percentage of area and as ratio between volatile peak area and internal standard peak area (ratio peak area/IS) are

shown in Table 19. Main chemical families of volatiles in cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract were aldehydes and carboxylic acids representing around 60% of total area volatiles in cooked hams. Apparently, cooked ham flavour results mainly from lipids oxidation and degradation during cooking.

Aromatic compounds were the third main chemical family of volatiles in cooked ham varying between 19.5% to 25.0% in cooked ham added with 1% acetate buffer and between 18.8% to 28.2% in cooked ham added with 1% spent yeast extract. Also sulphur compounds were detected in relative areas of around 8% to 14% in cooked ham added with 1% acetate buffer and 7% to 10% in cooked ham added with 1% spent yeast extract. These compounds derive from proteolysis of proteins and amino acids degradation and therefore being part of cooked ham flavour.

Esters were a minor group and varied between 1.4% to 4.8% in cooked ham added with 1% acetate buffer and between 2.2% to 3.8% in cooked ham added with 1% spent yeast extract.

Terpens were found between 3-11% in cooked ham added with acetate buffer and 4-9% in cooked ham added with spent yeast extract.

Table 19: Volatile compounds of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract at different cooking times after 12 days of production

	Cooked ham added with acetate buffer								Cooked ham added with spent yeast extract							
Volatile compounds	**1.5 h**		**2 h**		**2.5 h**		**3 h**		**1.5 h**		**2 h**		**2.5 h**		**3 h**	
Aldehyde	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**
Hexanal	1.56	0.006	1.65	0.011	2.52	0.015	2.22	0.009	1.35	0.007	1.47	0.008	1.02	0.010	1.86	0.014
Benzaldehyde	3.50	0.014	1.75	0.012	2.42	0.014	2.12	0.011	1.63	0.008	2.07	0.011	2.44	0.029	3.78	0.027
Octanal	1.41	0.006	1.68	0.011	1.64	0.010	0.94	0.004	1.00	0.005	1.49	0.008	0.69	0.007	1.49	0.012
Nonanal	10.63	0.041	16.35	0.111	10.83	0.064	10.04	0.041	14.63	0.075	16.79	0.092	14.72	0.178	21.60	0.178
Decanal	16.75	0.069	8.20	0.056	8.56	0.050	10.67	0.039	11.36	0.058	10.52	0.057	15.24	0.177	14.43	0.111
Dodecanal	0.55	0.002	0.00	0.000	0.00	0.000	0.74	0.002	0.11	0.001	0.32	0.002	0.00	0.000	0.00	0.000
Carboxylic acids																
Hexanoic acid	9.56	0.041	10.80	0.073	11.14	0.066	15.04	0.068	10.39	0.054	7.37	0.041	5.28	0.063	6.52	0.048
Octanoic acid	15.98	0.059	16.00	0.109	15.20	0.088	17.67	0.079	22.16	0.114	12.12	0.067	13.78	0.149	14.81	0.103
Nonanoic acid	3.09	0.013	2.01	0.014	1.86	0.007	0.00	0.000	0.74	0.004	2.37	0.013	0.00	0.000	1.61	0.015
Decanoic acid	0.00	0.000	1.16	0.008	1.37	0.005	0.00	0.000	0.00	0.000	0.77	0.005	0.00	0.000	0.00	0.000
Dodecanoic acid	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
Ester																
Octanoic acid methyl ester	1.03	0.004	0.49	0.003	0.08	0.001	0.35	0.001	0.51	0.003	0.09	0.001	0.00	0.000	0.00	0.000
Dodecanoic acid methyl ester	1.32	0.005	0.32	0.002	0.19	0.001	0.63	0.002	1.53	0.008	0.34	0.002	0.59	0.006	1.18	0.010
Hexadecanoic acid methyl ester	2.48	0.010	1.35	0.009	1.09	0.006	1.83	0.005	1.15	0.006	1.79	0.010	1.59	0.017	2.59	0.021
Aromatic																
Toluene	1.54	0.006	1.52	0.010	1.49	0.009	1.91	0.008	1.16	0.006	0.96	0.005	1.41	0.016	1.04	0.008
Ethylbenzene	2.24	0.009	3.13	0.021	2.96	0.017	2.32	0.011	2.24	0.011	3.51	0.019	3.15	0.032	1.96	0.015
p-Hylene	15.74	0.063	20.32	0.138	18.06	0.105	15.91	0.071	15.69	0.080	22.79	0.124	23.63	0.246	15.78	0.121
Terpens																
Limonene	2.09	0.008	4.98	0.034	10.86	0.064	2.39	0.010	3.70	0.019	5.84	0.032	7.75	0.094	2.36	0.017
Terpineol	0.99	0.004	0.36	0.002	0.46	0.003	0.64	0.003	0.83	0.004	1.36	0.008	1.39	0.017	1.15	0.009
Sulphur																
1-methylthio-1-propene (E)	5.48	0.024	5.54	0.038	6.90	0.039	10.29	0.043	6.86	0.035	5.70	0.031	5.11	0.052	5.46	0.040
1-methylthio-1-propene (Z)	4.11	0.016	2.43	0.016	2.40	0.013	4.32	0.018	3.04	0.016	2.40	0.013	2.25	0.023	2.35	0.018

RA%: Relative peak area expressed as % of total area of volatile compounds; pA/IS: ratio between arbitrary units of peak area and internal standard peak area. Sample n=4 for each cooking time.

Main effects of yeast extract addition and cooking times on quantitative profile of volatile compounds in cooked hams were evaluated by Two-Way ANOVA using as variables the ratio peak area/IS, *F* values and significance are detailed in Table 20. Aldehydes were the group that was more affected by both variables and with the exception of nonanal, aldehydes were significantly influenced by the interaction of yeast addition and cooking time.

Table 20: Two-way ANOVA results of yeast addition and cooking time effects on volatiles profile of cooked ham added with 1% acetate buffer and cooked ham added 1% spent yeast extract

	Effects of yeast addition		**Effects of cooking time**		**Interaction**	
Volatile compounds	***F***	***p***	***F***	***p***	***F***	***p***
Aldehydes						
Hexanal	0.93	0.344	12.12	0.000	7.11	0.001
Benzaldehyde	7.27	0.013	4.10	0.018	5.48	0.005
Octanal	0.49	0.490	2.16	0.118	4.71	0.010
Nonanal	7.77	0.010	2.12	0.124	2.47	0.086
Decanal	12.84	0.001	4.45	0.013	5.80	0.004
Dodecanal	4.81	0.038	6.14	0.003	13.54	0.000
Carboxylic acids						
Hexanoic acid	2.95	0.099	1.28	0.301	2.59	0.076
Octanoic acid	6.67	0.016	4.36	0.014	6.74	0.002
Nonanoic acid	2.55	0.130	2.29	0.117	2.18	0.159
Decanoic acid	1.36	0.272	0.81	0.389	----	----
Dodecanoic acid						
Esters						
Octanoic acid methyl ester	90.52	0.000	65.32	0.000	8.87	0.008
Dodecanoic acid methyl ester	6.80	0.016	2.38	0.096	1.60	0.218
Hexadecanoic acid methyl ester	12.22	0.002	2.10	0.126	7.57	0.001
Aromatics						
Toluene	0.24	0.624	6.03	0.003	5.46	0.005
Ethylbenzene	5.74	0.025	10.02	0.000	3.14	0.044
pXylene	13.42	0.001	11.81	0.000	6.27	0.003
Terpens						
DLimonene	1.65	0.210	7.16	0.001	2.15	0.120
Terpineol	17.00	0.000	2.01	0.139	3.59	0.028
Sulphur						
1-methylthio-1-propene (E)	1.05	0.315	4.79	0.009	1.86	0.162
1-methylthio-1-propene (Z)	1.94	0.176	4.53	0.012	8.94	0.000

Concerning carboxylic acids, octanoic acid was the only volatile compound to be significantly affected by yeast addition (F=6.67; p=0.016) and cooking time (F= 4.36; p=0.014) and by the interaction of both variables (F=6.74; p=0.002). Yeast extract addition presented significant effects in esters, octanoic acid methyl ester (F= 90.52; p=0.000),

decanoic acid methyl ester (F=6.80; p=0.016) and hexadecanoic acid methyl esters (F=12.22; p=0.002). The influence of cooking time was significant for octanoic acid methyl ester (F=65.32; p=0.000).

All aromatics compounds were affected by cooking time and the interaction of yeast addition and cooking time while ethylbenzene (F= 5.74; p=0.025) and p-hylene (F=13.42; p=0.001) were also affected by yeast addition.

Terpineol was significantly affected by processing method (F=17.00; p=0.000) and interaction between the two variables (F=3.59; p=0.028). Limonene was only affected by cooking time (F=7.16; p=0.001).

Sulphur compounds were not affected by yeast addition but cooking time had a significant effect on their formation and 1-methylthio-1-propene (Z) was also affected by the interaction of both: effect of yeast addition and cooking time.

To sum up, the effects of spent yeast extract addition and cooking time on characteristics of cooked ham highlight that the influence of spent yeast addition was prominent on physical parameters (mainly, L*, b*, hardness, gumminess and chewiness) when compared with the influence of cooking time. Concerning chemical parameters, the influence of spent yeast addition was also prominent when compared with the influence of cooking time. Cooked ham added with spent yeast extract presented increased ash and protein contents. Additionally, the relative proportion of polypeptides and proteins with molecular weight <14 kDa from PSDB protein fraction was higher, indicating yeast polypeptides and proteins that are hydrolysed during ham cooking are also extracted in this fraction. No significant effects of cooking time were found in this protein fraction. Concerning myofibrillar proteins (PSSIB fraction) the effects of yeast addition and cooking time were not significant. Some FAA, namely, Ala, Gly, Val, Ser, Thr, Lys, Trp were higher in cooked ham added with spent yeast extract, due to yeast FAA composition. Qualitative volatile profile of control cooked ham and cooked ham added with spent yeast extract was similar. Main chemical families of volatile compounds were aldehydes and carboxylic acids. However, quantitative differences were observed in some volatiles when evaluated as ratio between volatile peak area and internal standard peak area.

10.3. Characterization of cooked ham added with acetate buffer and cooked ham added with spent yeast extract after shelf-life period

Moisture and pH were analysed at end of shelf life in cooked ham added with acetate buffer and in cooked ham added with spent yeast extract and a *t*-Test was performed to observe if occurred changes of these parameters. Moisture content and pH values in cooked ham added with acetate buffer ranged from 72.9 to 76.3% and 6.21 to 6.42, respectively. In cooked ham added with spent yeast extract moisture content and pH values ranged from 72.3 to 74.9% and 5.25 to 6.51, respectively. The observed values oscillated for the different cooking times without an increasing or decreasing trend. With some exceptions, during shelf life periods, the moisture content and pH value suffered significant differences ($p<0.05$) in both processing methods when compared with values of 12 days of storage.

10.3.1. CIE Lab and TPA analysis

Colour and texture attributes at the end of shelf life (90 days) are shown in Table 21. Comparison between results obtained after 12 and 90 days of ham production was performed by *t*-Test to understand the modifications that occurred in colour and texture of each batch of cooked ham and respective cooking time (Table 22).

Table 21: CIE lab and TPA attributes of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract for each cooking time analysed after 90 days of production

	Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
	1.5 h	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
*L**	58.8±1.82	61.00±0.81	62.5±1.51	61.1±3.93	59.6±3.25	64.8±1.99	61.9±4.27	60.3±2.90
*a**	14.6±1.41	12.1±0.04	12.7±0.60	12.8±2.09	14.5±1.93	11.8±1.34	13.9±1.76	13.9±1.53
*b**	8.25±0.53	8.01±0.32	8.97±0.56	8.47±1.18	7.79±0.56	8.01±0.34	8.56±0.81	8.90±1.00
Hardness (N)	42.7±8.44	63.4±9.56	41.6±10.4	27.9±7.04	56.5±8.76	58.6±6.94	51.2±6.36	44.6±8.01
Springiness (mm)	1.24±0.02	1.22±0.00	1.21±0.05	1.31±0.05	1.29±0.05	1.26±0.03	1.26±0.04	1.27±0.02
Cohesiveness (-)	0.91±0.01	0.93±0.01	0.90±0.06	0.92±0.01	0.89±0.04	0.92±0.02	0.92±0.02	0.91±0.03
Gumminess (N)	38.7±7.18	58.7±8.38	37.6±10.4	25.7±6.32	50.1±6.16	53.6±6.22	46.9±4.84	40.5±6.49
Chewiness (Nmm)	48.2±9.23	64.8±12.0	45.5±13.2	33.7±8.60	64.7±8.15	67.9±8.72	59.3±7.56	51.3±8.52

Results presented in Mean ± standard deviation. Samples n=4 for each cooking time.

In general no significant differences were observed on colour and TPA results at 12 and 90 days for cooked ham added with 1% acetate buffer and cooked ham added 1% with spent yeast extract, however, some exceptions were observed (p<0.05).

Table 22: *t*-Test *p* values between CIE Lab and TPA parameters results obtained at 12 and 90 days for each cooked ham and respective cooking time

	Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
	1.5 h	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
*L**	0.034	0.751	0.060	0.952	0.001	0.071	0.557	0.744
*a**	0.001	0.560	0.805	0.721	0.001	0.683	0.014	0.441
*b**	0.081	0.067	0.362	0.244	0.675	0.781	0.141	0.392
Hardness (N)	0.812	0.140	0.039	0.002	0.866	0.697	0.127	0.052
Springiness (mm)	0.092	0.000	0.365	0.564	0.293	0.793	0.005	0.014
Cohesiveness (-)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gumminess (N)	0.147	0.002	0.184	0.111	0.027	0.002	0.194	0.075
Chewiness (Nmm)	0.304	0.004	0.351	0.132	0.123	0.001	0.725	0.127

Concerning texture profile, hardness of cooked ham added with 1% acetate buffer was only modified in 2 h and 3 h of cooking time while in cooked ham added with 1% spent yeast extract this attribute did not suffer significant modification after shelf life. Springiness, Gumminess and Chewiness presented significant modification only for 2 h in cooking ham added with 1% acetate buffer. Springiness of cooked ham added with 1% spent yeast extracts presented significant differences at 2.5 h and 3 h. Cohesiveness presented significant differences after shelf life in all cooking times and in both processing methods.

10.3.2. Protein degradation and peptides formation

Relative peak area expressed as percentage of PSDB and PSSIB protein fractions of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract with different cooking times analysed at the end of shelf life (90 days) are summarized in Table 23. As observed after 12 days of production, main peak area was T_R 15-25 min in PSDB and PSSIB protein fractions.

Table 23: Relative peak area expressed as percentage of PSDB and PSSIB protein fractions of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract with different cooking times analysed after 90 days of production

	Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
	1.5 h	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
PSDB*								
T_R 5-15 min	6.1	6.8	7.8	3.8	9.7	9.7	3.9	1.2
T_R 15-25 min	57.7	72.6	71.1	75.2	76.5	74.3	70.9	82.5
T_R 25-33 min	36.2	20.6	21.1	21.0	13.8	16.0	25.2	16.3
PSSIB**								
T_R 5-15 min	----	----	----	----	----	----	----	----
T_R 15-25 min	73.7	77.9	78.9	87.2	75.8	87.6	61.9	92.4
T_R 25-33 min	26.3	22.1	21.1	12.8	24.2	12.4	38.1	7.6

*Results are presented as relative % of total peak area of PSDB protein fraction; ** Results are presented as relative % of total peak area of PSSIB protein fraction; Samples n=4 for each cooking time

Comparison between results obtained after 12 and 90 days of ham production was performed by *t*-Test to understand the proteolysis of each cooked ham and respective cooking time (Table 24). *t*-test indicates that cooked ham added with 1% acetate buffer did not suffer significant modification in PSDB and PSSIB protein fractions, whereas in cooked ham added with 1% spent yeast extract some changes were observed on PSDB and PSSID protein fractions.

Relative peak area of PSDB fraction with molecular weight <14 kDa (R_T 5-15 min) varied during shelf life decreasing significantly from approximately 13% in 12 days to 9.7% in 1.5h and 2h and 3.9% and 1.2% for 2.5h and 3h at 90 days. Proteins with molecular weight >14 kDa <60 kDa (R_T 15-25 min) varied significantly in all cooking times with the exception of 2h that maintained similar value at 90 days. Proteins with molecular weight >60 kDa (R_T 25-33 min) varied significantly except at 1.5 and 3h.

PSSID protein fraction did not suffer significant modifications in relative areas in all peak areas with the exception of 2 h of cooking time in cooked ham added with 1% spent yeast extract samples.

Table 24: *t*-Test *p* values between protein and peptide results obtained at 12 and 90 days for each cooked ham and respective cooking time

	Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
	1.5 h	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
PSDB								
T_R 5-15 min	0.651	0.174	0.559	0.088	0.064	0.016	0.001	0.010
T_R 15-25 min	0.958	0.242	0.053	0.206	0.000	0.986	0.009	0.016
T_R 25-33 min	0.996	0.160	0.047	0.326	0.046	0.020	0.000	0.309
PSSIB								
T_R 5-15 min	----	----	----	----	----	----	----	----
T_R 15-25 min	0.151	0.088	0.243	0.052	0.118	0.004	0.915	0.053
T_R 25-33 min	0.151	0.088	0.243	0.052	0.118	0.004	0.915	0.053

10.3.3. Quantification of free amino acids

FAA analysis was carried out for both processing methods at each cooking time after 90 days of production, the results are shown in Table 25. Met and Cys were lower than LOQ in cooked ham added with 1% acetate buffer and in cooked ham added with 1% spent yeast extract at different cooking times. Asp, Arg and Tyr were lower than LOD. Also, in cooked ham added with acetate buffer Thr was lower than LOQ. Typical FAA profile of cooked ham observed at 12 days of storage (Ile > Glu > Gln > Trp) was changed in both processing methods. At the end of 90 days the FAA profile in cooked hams added with acetate buffer was Ile > Glu > His > Phe with some exceptions. In the case of cooked ham added with spent yeast extract FAA profile was Ile > His > Glu > Phe. The most important observation is the increase in His in cooked hams from both processing methods probably promoted by proteolysis that occurred during shelf life period.

At 90 days the main FAA groups in both processing methods continued to be aliphatic FAA. Moreover, acidic FAA was the second main group in cooked ham added with acetate buffer as same as 12 days. While a different behaviour was observed in cooked ham added with spent yeast extract being basics FAA due to the notorious increase in His content.

Table 25: FAA content of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract at different cooking times analysed after 90 days of production[a]

		Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
FAA	**Ions**	**1.5 h**	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
Ala	158	9.52 ± 0.22	7.16 ± 0.01	6.96 ± 0.38	9.06 ± 0.12	9.19 ± 0.07	9.86 ± 0.20	13.7 ± 0.05	9.29 ± 0.21
Gly	218	2.73 ± 0.02	2.44 ± 0.03	1.90 ± 0.30	2.69 ± 0.02	3.42 ± 0.05	3.70 ± 0.02	4.99 ± 0.05	3.52 ± 0.14
Val	260	3.32 ± 0.00	2.21 ± 0.02	2.93 ± 0.04	2.68 ± 0.03	3.00 ± 0.01	3.23 ± 0.00	5.98 ± 0.14	3.11 ± 0.13
Leu	200	1.84 ± 0.00	1.13 ± 0.00	1.55 ± 0.04	1.30 ± 0.00	1.73 ± 0.01	1.84 ± 0.02	5.56 ± 0.05	1.93 ± 0.01
Ile	200	95.6 ± 11.0	87.8 ± 0.35	127 ± 0.22	106 ± 1.35	93.2 ± 1.31	99.9 ± 0.03	93.7 ± 0.04	88.4 ± 10.1
Pro	184	2.11 ± 0.02	2.06 ± 0.00	2.25 ± 0.04	2.52 ± 0.02	2.42 ± 0.00	2.55 ± 0.00	3.08 ± 0.05	2.59 ± 0.05
Phe	302	16.3 ± 0.03	8.17 ± 0.35	11.1 ± 1.14	12.7 ± 0.01	10.3 ± 0.02	12.3 ± 0.06	8.20 ± 0.14	13.4 ± 0.09
Met	218	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Ser	362	LOQ	1.12 ± 0.00	1.84 ± 0.14	1.15 ± 0.00	2.61 ± 0.02	2.48 ± 0.00	4.09 ± 0.12	2.08 ± 0.02
Thr	404	LOQ	LOQ	LOQ	LOQ	1.59 ± 0.02	1.65 ± 0.03	3.24 ± 0.09	1.50 ± 0.05
Asp	302	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Hyp	388	2.39 ± 0.01	1.96 ± 0.00	LOQ	2.47 ± 0.04	2.08 ± 0.08	2.28 ± 0.00	2.29 ± 0.00	2.23 ± 0.08
Cys	406	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Glu	416	12.7 ± 7.35	22.1 ± 0.08	21.5 ± 2.07	29.9 ± 0.20	19.3 ± 1.73	23.1 ± 0.08	29.5 ± 0.12	18.1 ± 5.96
Arg	286	LOD	LOD	LOD	LOD	LOQ	LOD	LOD	LOD
Asn	417	LOD	0.90 ± 0.01	1.13 ± 0.01	1.09 ± 0.01	1.06 ± 0.00	1.17 ± 0.00	1.23 ± 0.01	1.07 ± 0.00
Lys	300	2.76 ± 0.09	2.83 ± 0.02	2.18 ± 0.24	3.42 ± 0.00	3.74 ± 0.04	3.68 ± 0.09	6.24 ± 0.01	3.63 ± 0.12
Gln	329	4.76 ± 0.43	LOD	2.46 ± 0.00	4.33 ± 0.03	6.23 ± 0.13	7.04 ± 0.38	12.4 ± 0.09	3.00 ± 0.09
His	196	13.0 ± 0.47	17.1 ± 0.21	LOQ	18.4 ± 0.28	23.2 ± 0.03	20.4 ± 0.00	31.5 ± 1.94	20.9 ± 0.76
Tyr	466, 302	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Trp	244	1.16 ± 0.01	2.49 ± 0.06	LOQ	1.69 ± 0.02	1.23 ± 0.01	1.41 ± 0.11	1.33 ± 0.00	1.58 ± 0.01
Ttoal FAA		166 ± 15.9	159 ± 0.47	183 ± 3.80	198 ± 0.70	194 ± 10.8	197 ± 0.60	134 ± 3.60	176 ± 3.60

[a] Concentration in mg/100g of dry matter, values are expressed in mean ± standard deviation; Sample n=4 for each cooking time. LOD= 0.32mg/100g (dw). LOQ= 1mg/100g (dw)

Table 26 presents *t*-Test *p* values between FAA results obtained at 12 and 90 days for each cooked ham and respective cooking time. Changes in FAA content were observed in cooked hams produced with addition of 1% acetate buffer and also in cooked hams produced with 1% spent yeast extract. Some FAA presented a decrease in their concentrations at 90 days in both processing methods. Most prominent decrease was observed for Ile and Trp, whereas an increase was observed for His. As it was described before, the reduction in some FAA could promote an increase in the production of volatile compounds, such as aldehydes (Ma, Hamid et al. 2012). The less abundant FAA has not suffered significant modifications in their concentrations during shelf life period.

Table 26: *t*-Test *p* values between FAA results obtained at 12 and 90 days for each cooked ham and respective cooking time

	Cooked ham added with acetate buffer				Cooked ham added with spent yeast extract			
FAA	**1.5 h**	**2 h**	**2.5 h**	**3 h**	**1.5 h**	**2 h**	**2.5 h**	**3 h**
Ala	0.000	0.824	0.002	0.542	0.750	0.131	0.003	0.002
Gly	0.000	0.206	0.004	0.632	0.019	0.277	0.000	0.799
Val	0.001	0.119	0.789	0.015	0.581	0.152	0.000	0.343
Leu	0.041	0.010	0.058	0.000	0.002	0.001	0.000	0.063
Ile	0.014	0.001	0.141	0.769	0.000	0.002	0.000	0.256
Pro	0.752	0.252	0.001	0.643	0.732	0.834	0.000	0.602
Phe	0.000	0.448	0.471	0.003	0.181	0.473	0.001	0.331
Ser	0.000	0.008	0.152	0.000	0.003	0.030	0.049	0.024
Thr	----	----	----	----	0.037	0.002	0.014	0.036
Hyp	0.000	0.018	----	0.014	0.030	0.000	0.000	0.007
Glu	0.084	0.372	0.006	0.059	0.006	0.062	0.000	0.204
Asn	0.000	0.042	0.001	0.000	0.008	0.580	0.019	0.002
Lys	0.255	0.008	0.010	0.507	0.000	0.188	0.006	0.048
Gln	0.234	0.128	0.000	0.404	0.047	0.201	0.020	0.000
His	0.000	0.000	----	0.000	0.000	0.000	0.000	0.000
Trp	0.124	0.000	0.021	0.284	0.288	0.000	0.000	0.000
Ttoal FAA	0.005	0.181	0.002	0.061	0.032	0.209	0.005	0.750

Total FAA decreased from 12 days to 90 days, however this decrease was significant only for 1.5 h and 2.5 h of cooking time.

10.3.4. Volatile compounds profile

Volatile profile of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract at different cooking times were assayed after 90 days of production. The relative areas expressed as % and ratio between peak area of each volatile compound and internal standard are shown in Table 27.

Main chemical families of volatile compounds in cooked ham added with 1% acetate buffer and in cooked ham added with 1% spent yeast extract were aldehydes, with around 30% of total peak areas, followed by terpens in cooked ham added with 1% acetate buffer and by aromatics in cooked ham added with spent yeast extract. These two groups represented approximately 20% of total peak area of ham volatiles from each processing

method. Carboxylic acids represent around 15% of total volatile peak area in cooked hams, and were the third main group in cooked hams from both processing methods.

Relative areas of sulphur compounds were around 6% in cooked ham added with 1% acetate buffer and 4% in cooked ham added with 1% spent yeast extract. These compounds derive from proteolysis of proteins and amino acids degradation being part of cooked ham flavour. Esters were the minorities group with around 5% of total volatile peak area in cooked ham added with acetate buffer and cooked ham added with spent yeast extract.

Esters in cooked ham added with acetate buffer, increased during shelf life. In cooked ham added with spent yeast extract, the relative peak area of esters decreased after shelf life period. This group is the minor group expressed as relative peak area and varied between 2.3% to 8.7% in cooked ham added with acetate buffer and between 1.7% to 9.3% in cooked ham added with spent yeast extract.

Total relative area of aromatic compounds group decreased at the end of shelf life period and varied between 11.5% to 20.7% for cooked ham added with acetate buffer and between 5.8% to 21.4% for cooked ham added with spent yeast extract. Sulphur compounds did not present considerable variation and relative areas were between 2.9 to 7.6% in cooked ham added with acetate buffer and 2.9 to 6.2% in spent yeast extract.

Terpens group increased during shelf life period varying between 10.4% to 26.7% in cooked ham added with 1% acetate buffer and 4.4% to 17.4% in cooked ham added with 1% spent yeast extract. In cooked ham added with 1% acetate buffer it was the second main group.

At 90 days aldehydes increased considerably the relative % of area in cooked with and without spent yeast extract, from around 30% at 12 days to around 40% at the end of shelf life. In opposite carboxylic acids decreased the relative % of areas in cooked of both processing methods from around 30% to 15%.

Table 28 presents *t*-test *p* values between volatile profile results of the ratio pA/IS obtained at 12 and 90 days for each cooked ham and respective cooking time, to study the evolution observed in cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extracts with different cooking times at the end of shelf life period.

Dodecanal increased significantly in all cooking times. Other aldehydes also increased with some exceptions. Decanal was the unique aldehyde that in all cooking times did not vary significantly the ratio pA/IS during storage time. The ratio pA/IS of dodecanoic and decanoic acids increased significantly in all cooking times.

Esters compounds increased the ratio pA/IS, however the increase was significant only in cooked ham added with 1% acetate buffer. Most of the changes in the group described before were due the autoxidation of free fatty acids during storage time as described previously for conventional samples.

Terpineol increased in samples of both processing methods and limonene increased at the end of shelf life in cooked ham added with acetate buffer. However, in cooked ham added with spent yeast extracts limonene maintained its proportion.

Table 27: Volatile compounds of cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract at different cooking times after 90 days of production

	Cooked ham added with acetate buffer								Cooked ham added with spent yeast extract							
Volatile compounds	**1.5 h**		**2 h**		**2.5 h**		**3 h**		**1.5 h**		**2 h**		**2.5 h**		**3 h**	
Adehydes	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**	**RA%**	**pA/IS**
Hexanal	1.65	0.014	3.13	0.038	1.88	0.023	1.80	0.051	1.23	0.016	3.05	0.031	1.37	0.037	1.61	0.052
Benzaldehyde	2.46	0.021	2.60	0.032	2.37	0.029	1.23	0.062	2.27	0.030	2.86	0.038	5.74	0.168	2.05	0.042
Octanal	1.01	0.009	3.18	0.042	1.67	0.020	6.52	0.186	3.12	0.041	4.50	0.050	0.86	0.022	0.94	0.034
Nonanal	17.91	0.156	20.25	0.263	13.59	0.167	50.98	1.496	30.05	0.397	35.24	0.391	36.63	0.229	14.17	0.337
Decanal	4.67	0.040	3.00	0.037	3.64	0.045	2.47	0.091	5.08	0.067	4.82	0.055	1.54	0.066	2.87	0.108
Dodecanal	1.64	0.014	1.63	0.020	1.39	0.017	0.77	0.027	1.67	0.022	1.45	0.018	0.74	0.019	1.16	0.035
Carboxylic acids																
Hexanoic acid	4.87	0.041	3.72	0.047	5.75	0.071	2.77	0.088	3.03	0.040	5.81	0.076	3.54	0.113	3.30	0.104
Octanoic acid	9.14	0.080	5.55	0.066	10.77	0.133	4.32	0.174	5.30	0.069	5.67	0.123	13.76	0.288	7.02	0.173
Nonanoic acid	0.96	0.008	0.51	0.006	0.42	0.005	0.34	0.012	0.78	0.010	0.63	0.011	1.27	0.022	1.03	0.011
Decanoic acid	2.24	0.019	0.88	0.011	1.43	0.018	0.72	0.023	0.95	0.013	0.96	0.013	0.70	0.027	0.85	0.022
Dodecanoic acid	2.75	0.024	0.66	0.009	1.14	0.014	0.90	0.026	0.98	0.013	1.21	0.016	4.00	0.024	0.62	0.017
Esters																
Octanoic acid methyl ester	0.98	0.008	0.71	0.009	0.45	0.006	0.17	0.01	0.24	0.003	0.22	0.003	8.26	0.386	1.34	0.002
Dodecanoic acid methyl ester	2.60	0.023	2.82	0.035	6.75	0.083	1.35	0.040	0.32	0.004	0.21	0.018	0.57	0.207	2.76	0.014
hexadecanoic acid methyl ester	2.01	0.017	1.36	0.017	1.47	0.018	0.76	0.025	1.23	0.016	1.36	0.023	0.48	0.022	0.83	0.027
Aromatics																
Toluene	1.57	0.014	1.17	0.014	0.92	0.011	0.44	0.029	0.95	0.013	1.33	0.015	0.65	0.046	1.14	0.020
Ethylbenzene	14.19	0.124	9.78	0.119	8.57	0.105	7.69	0.383	13.93	0.184	13.90	0.166	3.52	0.227	9.76	0.261
pHylene	4.90	0.044	4.76	0.057	4.33	0.053	3.42	0.164	6.20	0.082	6.20	0.078	1.63	0.094	4.44	0.115
Terpens																
Limonene	13.74	0.118	18.95	0.231	19.18	0.235	8.26	0.244	13.03	0.172	2.19	0.082	3.78	0.109	9.56	0.302
Terpineol	3.62	0.031	7.83	0.096	7.31	0.090	2.18	0.062	4.32	0.057	2.19	0.044	7.96	0.042	4.43	0.062
Sulphur																
1-methylthio-1-propene (E)	4.77	0.041	5.06	0.061	4.65	0.057	1.99	0.055	3.55	0.047	4.17	0.048	2.17	0.054	2.04	0.074
1-methylthio-1-propene (Z)	2.32	0.020	2.50	0.031	2.34	0.029	0.94	0.027	1.79	0.024	2.07	0.023	0.85	0.023	0.85	0.031

RA%: Relative peak area expressed as % of total area of volatile compounds; pA/IS: ratio between arbitrary units of peak area and internal standard peak area. Sample n=4 for each cooking time

Table 28: *t*-Test p-values of volatile compounds of cooked ham added with acetate buffer and spent yeast extract after shelf life performed with pA/IS.

	Cooked ham added with acetate buffer								Cooked ham added with spent yeast extract							
	1.5 h		2 h		2.5 h		3 h		1.5 h		2 h		2.5 h		3 h	
Adehydes	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Hexanal	16.35	0.010	17.96	0.000	0.12	0.003	1.38	0.000	32.00	0.001	4.13	0.003	7.75	0.077	5.85	0.001
Benzaldehyde	16.29	0.047	11.75	0.005	20.63	0.006	7.42	0.165	0.04	0.000	0.84	0.000	0.35	0.000	0.07	0.067
Octanal	4.10	0.025	195.56	0.082	0.26	0.004	9.97	0.000	6.86	0.026	5.97	0.000	63.69	0.009	38.91	0.009
Nonanal	141.93	0.002	2867.47	0.098	0.44	0.009	22.06	0.002	8.27	0.021	7.36	0.005	5.32	0.489	29.88	0.076
Decanal	6.30	0.068	0.55	0.054	1.58	0.245	7.62	0.094	0.03	0.347	3.08	0.719	3.82	0.092	2.47	0.877
Dodecanal	0.06	0.000	105.500	0.000	6.140	0.000	6.35	0.021	5.10	0.000	4.530	0.000	8.310	0.009	3.990	0.000
Carboxylic acids																
Hexanoic acid	1.61	0.963	6.36	0.035	2.10	0.456	0.49	0.150	2.87	0.397	0.38	0.003	0.03	0.158	1.71	0.009
Octanoic acid	0.06	0.182	0.75	0.012	2.34	0.154	0.10	0.000	33.73	0.075	4.00	0.222	2.40	0.172	1.94	0.218
Nonanoic acid	2.14	0.347	23.86	0.058	4.96	0.516	8.980	0.099	15.69	0.128	0.14	0.601	4.390	0.001	4.07	0.193
Decanoic acid	8.400	0.000	0.02	0.144	0.25	0.008	4.290	0.000	6.580	0.003	8.79	0.020	6.450	0.041	7.820	0.000
Dodecanoic acid	22.340	0.015	7.110	0.050	7.230	0.005	5.080	0.000	8.850	0.001	4.54	0.000	4.610	0.006	4.700	0.000
Esters																
Octanoic acid methyl ester	0.01	0.000	5.38	0.000	65.14	0.001	7.25	0.005	7.47	0.570	52.76	0.118	5.270	0.000	6.660	0.008
Dodecanoic acid methyl ester	12.51	0.009	20.50	0.009	2.43	0.000	4.89	0.000	4.69	0.004	2626.10	0.169	8.81	0.134	43.27	0.534
Hexadecanoic acid methyl ester	5.45	0.000	221.79	0.022	0.15	0.000	1.86	0.006	6.57	0.111	222.39	0.040	0.00	0.177	10.20	0.468
Aromatics																
Toluene	2.46	0.001	2.52	0.089	2.30	0.008	8.55	0.298	2.73	0.000	2.04	0.000	6.33	0.369	2.94	0.001
Ethylbenzene	4.78	0.000	14.97	0.003	2.82	0.000	8.75	0.114	5.59	0.000	0.15	0.000	8.07	0.232	3.11	0.000
pHylene	1.78	0.220	2.78	0.000	2.43	0.000	7.06	0.269	4.20	0.840	5.60	0.003	0.04	0.070	0.01	0.772
Terpens																
Limonene	4.53	0.000	17.59	0.002	542.43	0.818	5.75	0.000	6.83	0.010	1803.30	0.214	35.42	0.681	5.21	0.000
Terpineol	3.31	0.000	28.61	0.004	7.30	0.000	7.88	0.001	6.01	0.000	91.10	0.032	97.67	0.018	5.43	0.000
Sulphur																
1-methylthio-1-propene (E)	47.80	0.248	26.25	0.014	0.36	0.000	1.38	0.064	0.48	0.011	2.43	0.006	1.28	0.679	0.63	0.000
1-methylthio-1-propene (Z)	5.33	0.004	3.64	0.007	9.26	0.000	3.15	0.007	11.42	0.005	0.08	0.004	44.12	0.842	16.61	0.005

pA/IS: ratio between arbitrary units of peak area and internal standard peak area

10.4. Characteristics of cooked hams with reduced tumbling time

The cooked hams with reduced tumbling time with addition of 1% acetate buffer and with addition of 1% spent yeast extract differed significantly in some characteristics that were evaluated. Significant differences were observed in proximal composition (protein and ash content) due to the protein and intracellular components of *Saccharomyces* yeast extract. Although, no significant differences were observed for lipids, moisture, pH, Na^+ and K^+.

Significant differences in textual characteristics between cooked ham added with 1% acetate buffer and cooked ham added with 1% spent yeast extract were observed. For all cooking times the ham added with spent yeast extract presented higher hardness. This can be explained by the denaturation of proteins of spent yeast extract due to heat action that strength a complex gel and improve the textural characteristics of cooked ham.

Concerning protein degradation and peptides formation, the addition of spent yeast extract decreased the proportion of PSDB protein fraction while increased peptides proportion due to the formation of peptides from proteolysis of yeast proteins that results from cooking temperatures.

Volatile compounds were not significantly affected by the addition of spent yeast extract. Other studies describe the addition of yeast extract to improve flavour of fermented meat products. Campagnol, dos Santos et al. (2011) found that the addition of 2% of purified commercial yeast extract increased volatile compounds derived from amino acids and carbohydrate catabolism. This is justified because the product used by this author did not suffer critical thermal process but suffered action from microorganisms.

The addition of spent yeast extract did not modify the volatile profile of cooked ham because the volatile compounds of cooked ham added with 1% acetate buffer and added with 1% yeast extract were the same.

At 90 days cooked hams with spent yeast extract presented similar evolution as cooked ham added with acetate buffer. Significant modifications were observed in moisture content, pH and cohesiveness, whereas textural and colour characteristics, did not suffer significant modifications during storage period in a global overview. Additionally, in PSDB and in PSSIB protein fraction, no significant modifications were observed in cooked ham added with 1% spent yeast extract and cooked ham added with 1% acetate buffer.

FAA profile of cooked ham produced by both processing methods followed a similar trend between 12 and 90 days, although cooked ham added with spent yeast extract presented higher content of some FAA. After 90 days of production, the FAA profile suffers significant modification with an increase of His and a decrease of Gln and Trp but the increase in His was remarkable in cooked ham with spent yeast extract. A decrease was also observed in the content of major FAA such as Ile, Glu, Gln and Trp and thus in total FAA of cooked hams from both processing methods.

Similar evolution of volatile profile was observed at the end of shelf life in cooked ham produced by both processing methods.

Moisture, protein, lipids, FAA and volatiles from ham added with 1% acetate buffer and added with 1% spent yeast extract cooked during increasing periods oscillated significantly with a random variation. Thus, no advantages were observed on increasing cooking time.

To sum up, concerning the effect of shelf life, in general, no significant differences were observed on physical parameters for cooked hams added with 1% acetate buffer used as control and cooked hams added with 1% spent yeast extract when each cooking time was compared at 12 and 90 days. Regarding chemical parameters increased proteolysis, His, aldehydes and esters were observed at the end of shelf life.

Section C: Sensorial characteristics of cooked ham with and without spent *Saccharomyces* yeast extract: relation with its physical and chemical parameters

11. Sensorial characteristics of cooked ham with and without yeast extract

Cooked ham sensorial characteristics influence the acceptance or rejection by consumers. Sensorial analyses were performed in three different types of ham cooked during 1.5 h: *i)* with addition of 1% spent yeast extract and 9 h tumbling time, *ii)* without spent yeast extract and added only with the acetate buffer and 9 h tumbling time and *iii)* conventional ham with 18 h tumbling time. These samples were analysed by a sensory panel 12 and 90 days after production, the scores obtained by the sensory panel are presented in Tables 29 and 30, respectively.

ANOVA analyses performed to the results obtained after 12 days of production indicate no significant differences between the three types of ham concerning texture and flavour (Table 29). However, significant differences were observed between cooked ham with and without spent yeast extract with regard to colour attribute and between cooked ham with spent yeast extract and conventional cooked ham in global appreciation being the first one the lower scored by panellists. Although, the three types of ham had a global appreciation higher than 3, which is the minimum satisfactory level.

Table 29: Scores of sensory analysis of cooked hams with 1.5 h of cooking time after 12 days of production performed by a trained panel

	Cooked ham with spent yeast extract			Cooked ham with acetate buffer			Conventional Samples			
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	*p*-value
Texture (in mouth)	3.45 ± 0.93	2	5	4.45 ± 0.93	3	6	4.45 ± 1.03	3	6	NS
Colour	3.91 ± 0.83^{a}	3	5	4.72 ± 0.91^{b}	4	6	4.45 ± 0.52^{a,b}	4	5	<0.05
Flavour	3.54 ± 0.93	2	5	3.91 ± 1.13	2	6	4.36 ± 1.12	2	6	NS
Global appreciation	3.54 ± 0.85^{a}	2	5	4.09 ± 0.83^{a,b}	3	5	4.45 ± 0.69^{b}	3	5	<0.05

Scale used ranged from 1 to 6: 1 = Poor; 2 = Unsatisfactory; 3 = Satisfies; 4 = Good; 5 = Very Good; 6 = Excellent.. NS: No significant differences between type of ham by ANOVA and Tukey´s test. Different letter in same row represent significant differences between values.

Table 30 shows the scores of sensory attributes of the three types of cooked ham 90 days after production. ANOVA analyses indicate that no significant differences were observed between the three types of cooked ham concerning all variables analysed by panellists. All

cooked hams were scored by panellist with values higher than 4 with regard to global appreciation.

Table 30: Scores of sensory analysis of cooked hams with 1.5 h of cooking time after 90 days of production performed by a trained panel

	Cooked ham with spent yeast extract			Cooked ham with acetate buffer			Conventional Samples			
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	*p*-value
Texture (in mouth)	4.25 ± 1.29	2	6	4.00 ± 1.04	2	6	4.33 ± 1.03	2	6	NS
Colour	4.00 ± 1.04	2	5	4.42 ± 1.08	3	6	3.92 ± 0.79	3	6	NS
Flavour	3.75 ± 1.13	1	5	4.17 ± 1.19	2	6	4.17 ± 0.94	2	5	NS
Global appreciation	4.25 ± 0.97	2	5	4.08 ± 1.08	2	6	4.25 ± 1.06	2	6	NS

Scale used ranged from 1 to 6: 1 = Poor; 2 = Unsatisfactory; 3 = Satisfies; 4 = Good; 5 = Very Good; 6 = Excellent.. NS: No significant differences between type of ham by ANOVA and Tukey´s test. Different letter in same row represent significant differences between values.

Comparison between scores obtained for the three types of cooked hams at 12 days and 90 days are represented in Figure 18. After 12 days of production (Figure 18A) cooked ham added with spent yeast extract presented the lowest scores whereas cooked ham added with acetate buffer and conventional cooked ham shown similar scores, indicating that 9 h and 18 h of tumbling has not influenced significantly the sensorial characteristics of the final product. After 90 days of production the sensorial characteristics of cooked ham added with spent yeast extract were similar to those of cooked ham without spent yeast extract and with those of conventional ham (Figure 18B). At the end of shelf life all cooked hams presented scores around 4, which mean "Good" in the used scale.

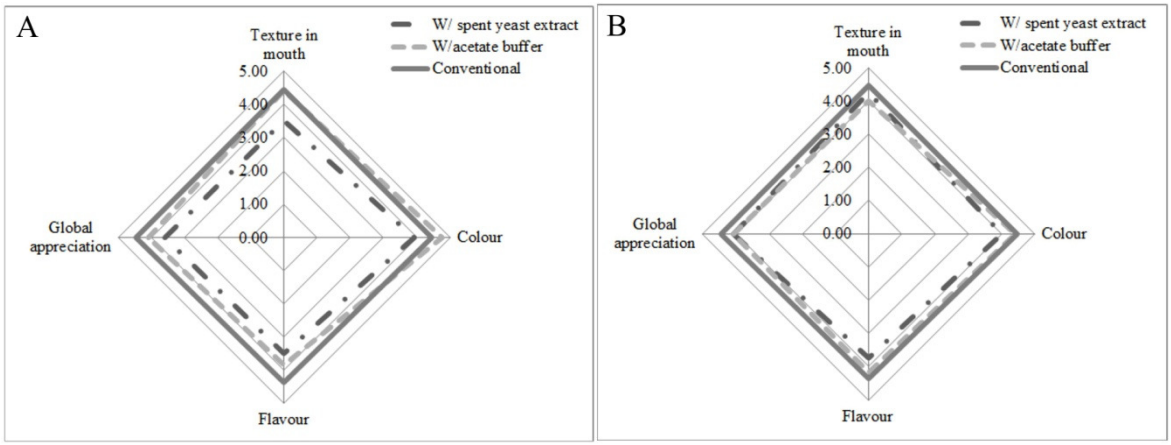

Figure 18: Sensory attributes of three types of cooked ham (with and without yeast extract and conventional) during shlef life. A: 12 days; B: 90 days after production

No relevant comments were made by panellists, the most relevant comment is that one of the panellists mentioned that at 12 days samples presented a more "plastic" texture that was attenuated after 90 days. Additionally, one panellist mentioned that at 12 day colour is pink and at 90 days is less intense. Concerning the preferred sample similar results were observed at 12 days and at 90 days, 2 panellists preferred cooked ham with addition of 1% spent yeast extract and 9 h tumbling time, 4 panellists preferred cooked ham without spent yeast extract and added only with the 1% acetate buffer and 9 h tumbling time and *5* panellists preferred the conventional ham with 18 h tumbling time.

11.1. Quality attributes related with mouth texture of cooked ham

Figure 19 shows TPA attributes of the three types of cooked hams analysed at 12 and 90 days. Similar results were observed between cooked ham added with spent yeast extract and conventional samples at 12 days while cooked hams added with acetate buffer presented significantly lower texture parameters (Figure 19A).

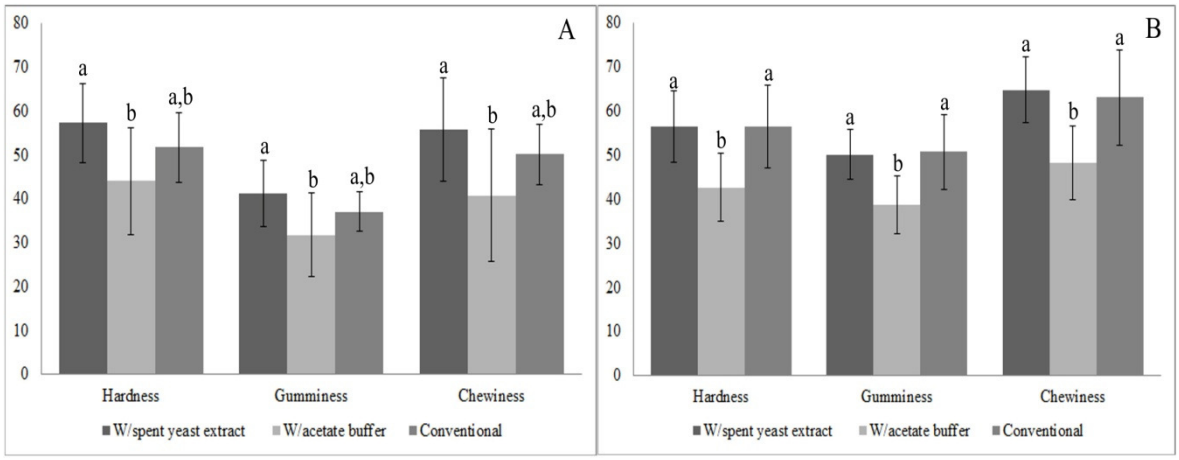

Figure 19: TPA of the three types of cooked hams. A) 12 days of storage; B) 90 days of storage.
Different letters in the same attribute for different types of cooked ham indicate significant differences between samples

The same behaviour was observed after 90 days of storage (Figure 19 B) since cooked ham added with spent yeast extract and conventional cooked ham presented similar texture and lower values were observed in cooked ham added with acetate buffer. These differences were significant for hardness, gumminess and chewiness. The results highlight that the tumbling time influences ham texture, but this effect can be reduced by yeast extract addition.

The scores obtained by Panellists for mouth texture evaluated the quality of texture, ranging from poor to excellent. No significant differences were found between the three types of ham indicating that the differences obtained in TPA values were not relevant for mouth texture appreciation.

11.2. Quality attributes related with sensorial colour of cooked ham

Figure 20 shows *L**, a* and *b** values, of the three types of cooked hams analysed at 12 and 90 days. Figure 20A indicates differences between CIE Lab parameters of the three types of cooked ham. The *L** value was significantly different ($p<0.05$) in cooked ham added with 1% yeast extract whereas cooked ham added with 1% acetate buffer and conventional cooked ham were similar. Concerning *a** value it was lower in cooked ham added with spent yeast extract than in the other two types of hams. *b** value presented similar value in cooked ham added with spent yeast extract and in conventional samples but it was significantly higher in cooked ham added with acetate buffer ($p<0.05$).

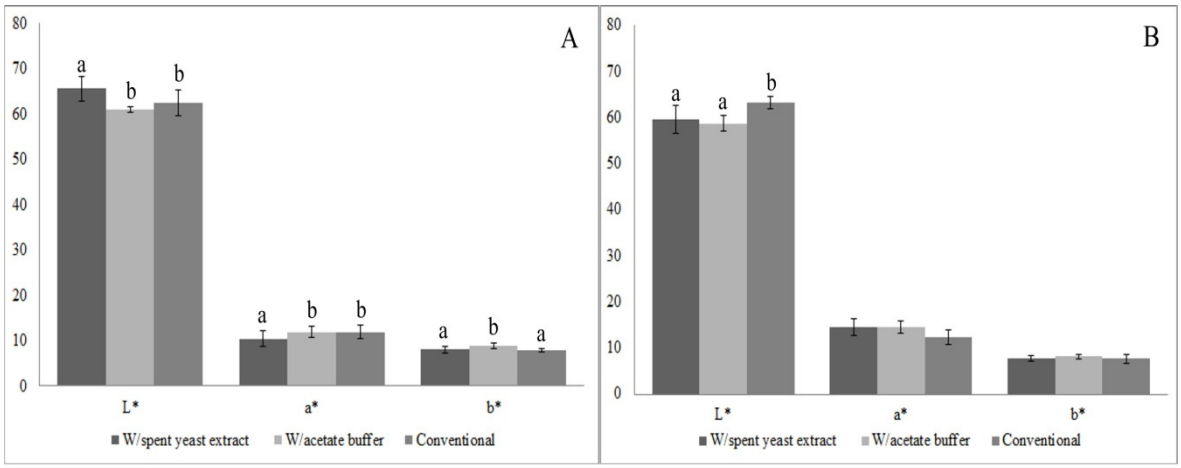

Figure 20: CIE *Lab* of the three types of cooked hams. A) 12 days of storage; B) 90 days of storage.
Different letters in the same attribute for different types of cooked ham indicate significant differences between samples

After shelf life period (Figure 20B) similar *L** values were observed in cooked ham added with 1% yeast extract and in cooked ham added with 1% acetate buffer whereas significantly higher ($p<0.05$) values were observed in conventional cooked ham. No significant differences were observed between the three types of ham for *b** and *a** values.

The scores of colour appreciation by panellists were significantly different in conventional samples and in cooked ham added with 1% acetate buffer, when compared with cooked ham added with 1% spent yeast extract at 12 days. No significant differences were observed at 90 days. These scores indicate that panellists appreciated ham samples with lower *L** and higher a* values. These results were not in agreement with Toldrá, Mora et al. (2010) that highlighted consumers preference by red and light colours. However, in general results from instrumental and sensorial evaluation were in agreement with each other.

11.3. Quality attributes related with flavour of cooked ham

Flavour of cooked ham is influenced by biochemical changes mainly due to enzymatic reactions, lipolysis and proteolysis that contribute to the formation of fatty acids and free amino acids, precursors of volatiles which have strong effects on taste and aroma. Thus, volatiles and FAA have a key role in flavour of cooked ham.

Cluster analysis was performed using as variables the ratio between peak area and internal standard of all volatile compounds of the three types of ham. The dendogram of cluster analysis is presented in Figure 21. Results highlight that at 12 days the volatile compounds of conventional cooked ham and cooked ham added with spent yeast extract presented similar characteristics, whereas cooked ham with acetate buffer presented differences although these samples are grouped in the same cluster. At 90 days volatiles compounds from conventional cooked ham and cooked ham added with spent yeast extract are grouped in one main cluster, denoting similar volatile profile at the end of shelf life. Moreover, cooked ham added with acetate buffer at 90 days is not included in this cluster but associated with cluster from 12 days.

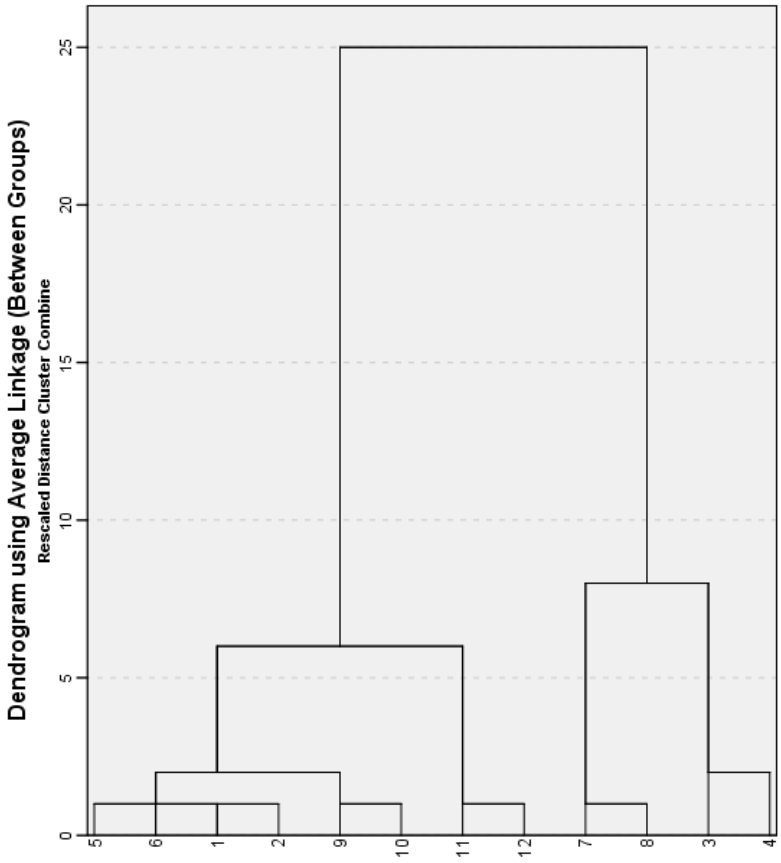

Figure 21: Dendogram of cluster analysis for volatile compounds in different process with 1.5 h of cooking time
Numbers 1 and 2 are from two batches of conventional cooked ham at 12 days, numbers 3 and 4 correspond to two batches of conventional cooked ham at 90 days. Numbers 5 and 6 correspond to cooked hams with spent yeast extract at 12 days and numbers 7 and 8 correspond cooked hams with spent yeast extract at 90 days. Cooked ham with acetate buffer are numbers 9 and 10 for 12 days and 11 and 12 for 90 days.

Another Cluster analysis was performed using as variables the content of FAA of the three types of ham. The dendogram of cluster analysis (Figure 22) presents two main clusters. One cluster grouped all the samples analysed at 12 days, where the other cluster grouped the samples analysed at 90 days, highlighting that changes were observed on FAA between 12 and 90 days, however the pattern is the same.

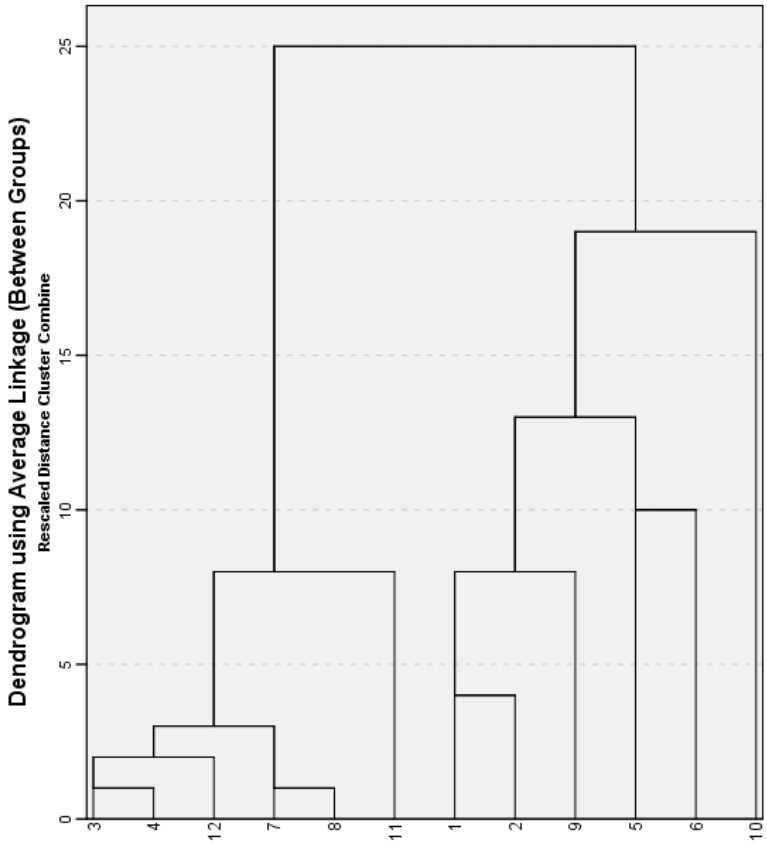

Figure 22: Dendogram of cluster analysis for FAA content in different process with 1.5 h of cooking time

Numbers 1 and 2 are from two batches of conventional cooked ham at 12 days, numbers 3 and 4 correspond to two batches of conventional cooked ham at 90 days. Numbers 5 and 6 correspond to cooked hams with spent yeast extract at 12 days and numbers 7 and 8 correspond cooked hams with spent yeast extract at 90 days. Cooked ham with acetate buffer are numbers 9 and 10 for 12 days and 11 and 12 for 90 days

The two dendograms obtained show that tumbling time and yeast addition promoted slight differences on volatile and FAA profiles whereas the shelf-life clearly influenced these chemical parameters.

The scores of flavour appreciation by panellists were not significantly different in the three types of ham at 12 days. The same was observed at 90 days. Thus, the differences highlighted by cluster analyses for chemical composition were not perceived by panellists.

11.4. Quality attributes related with global appreciation

With regard to sensory attributes global appreciation of cooked ham at 12 and 90 days was significantly correlated with mouth texture (r=0.78, p<0.01), colour (r=0.649, p<0.01) and

flavour (r=0.770, p<0.01). Negative correlations were observed between scores of global appreciation and texture parameters, such as, hardness (r=-0.303, p<0.05), gumminess (r=-0.322, p<0.01), and chewiness (r=-0.350, p<0.01). No significant correlations were found between global appreciation and other physical or chemical parameters.

Principal component analysis (PCA) was performed using as variables global appreciation and all the studied physical and chemical parameters that presented significant differences between the three different types of ham cooked during 1.5 h (with addition of 1% spent yeast extract and 9 h tumbling time; with addition of 1% acetate buffer and 9 h tumbling time and conventional ham with 18 h tumbling time) and also the variables that presented significant differences between 12 and 90 days, to reduce the dimensionality of the data and pinpoint the most important factors causing variability. The variables selected for this PCA analyses were global appreciation, hardness, gumminess, chewiness, L^*, b^*, Ala, Gly, Val, Leu, Iso, Ser, Thr, Glu, His, Try, <14 kDa PSDB protein fraction, >60 kDa PSSIB protein fraction, octanoic acid methyl ester, nonanal, octanoic acid, decanoic acid, dodecanoic acid methyl ester, hexadecanoic acid methyl ester, and octanoic acid methyl ester. The PCA resulted in six principal components with eigenvalues higher than 1.0 that justified 87.13% of data variance. The PCA results are shown in Table 31 and Figure 23. The first component (Component 1) by itself condensed 30.66% and is associated with the effect of shelf life on cooked ham, whereas the second component (Component 2) represented 18.94% of the total information and is associated with changes that occur due to tumbling time and yeast extract addition. The plot of Figure 23 show that at 12 days the addition of yeast extract to cooked ham tumbled during 9 h changed its texture and composition when compared with the same cooked ham without yeast extract addition and lead to similar characteristics of the conventional cooked ham tumbled during 18 h. After 90 days physical and chemical characteristics of cooked ham changed when compared with these attributes at 12 days, but lower differences were observed between the three types of ham.

Table 31: Principal components (PC) loadings for global appreciation and selected physical and chemical characteristics

	Matrix component					
1		2	3	4	5	6
Global appreciation	-0,028	-0,115	0,63	-0,007	0,382	-0,077
Hardness	0,168	0,562	-0,601	0,287	0,148	0,334
Gumminess	-0,267	0,702	-0,545	0,224	0,114	0,227
Chewiness	-0,128	0,687	-0,578	0,21	0,109	0,208
*L**	0,531	0,221	0,028	0,439	-0,302	-0,288
*b**	-0,082	-0,523	-0,195	0,079	-0,139	0,346
Ala	0,14	0,764	0,28	-0,403	-0,031	0,224
Gly	0,112	0,914	0,097	-0,137	0,218	-0,088
Val	-0,079	0,812	0,223	-0,284	-0,394	0,124
Leu	0,546	0,615	0,122	-0,253	-0,394	0,093
Iso	0,5	-0,392	0,358	0,336	0,468	0,137
Ser	0,806	0,431	-0,176	0,063	0,162	-0,253
Thr	0,626	0,718	-0,114	-0,031	0,09	-0,203
Glu	0,663	-0,259	-0,165	0,45	0,059	-0,213
Lys	0,729	0,632	0,157	-0,05	0,119	0,002
Glu	0,799	0,124	0,302	-0,18	0,298	0,276
His	-0,772	0,526	-0,148	-0,09	0,06	-0,228
Try	0,956	0,096	0,142	-0,068	-0,06	0,148
<14 kDa PSDB	0,657	0,506	-0,035	-0,133	0,104	-0,134
>60 kDa PSSIB	0,625	-0,132	-0,088	0,058	-0,537	-0,147
Nonanal	-0,601	0,585	-0,204	-0,116	0,203	-0,371
Octanoic acid methyl ester	-0,531	0,416	0,432	0,549	-0,109	0,067
Octanoic acid	-0,013	0,651	0,334	0,608	-0,039	-0,028
Decanoic acid	-0,671	0,591	0,359	-0,014	0,005	0,129
Dodecanoic acid methyl ester	-0,495	0,471	0,447	0,486	-0,184	0,076
Hexadecanoic acid methyl ester	-0,812	0,218	0,051	-0,232	0,117	-0,158

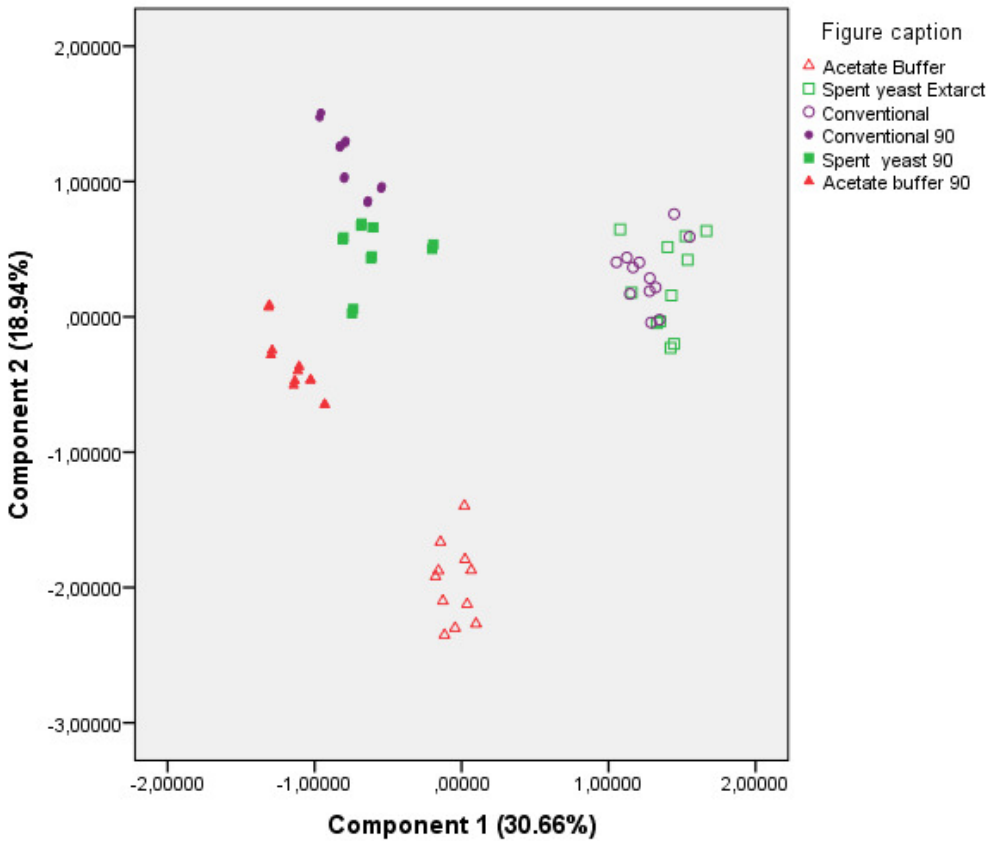

Figure 23: Projection of samples in the two main Components (PC1 and PC2), representing 59.60% of total variance

PCA highlights that the addition of yeast extract increased cooked ham hardness, gumminess, chewiness, FAA, <14 kDa PSDB protein fraction, and volatiles. The shelf life increased volatiles, except octanoic acid and decreased FAA, except His and decreased >60 kDa PSSIB protein fraction as consequence of the occurrence of proteolysis.

The loadings of global appreciation of cooked ham at 12 and 90 days were not significant on Component 1 and on Component 2. Thus, the physical and chemical modifications that take place due to yeast extract addition, tumbling time or shelf life have not a significant impact on global appreciation of cooked ham.

Chapter 4.

Conclusions

Conclusions

The work described in this thesis was performed to found a new application to spent S*accharomyces* yeast extract as a food ingredient obtained from a by-product of the Brewing industry. This new ingredient can be used to reduce tumbling time in cooked ham production maintaining the physical, chemical and sensorial characteristics of the conventional cooked ham elaborated by a local meat industry.

Proximate composition of cooked hams used in this study was similar to the literature. Comparison between conventional cooked ham (18 h of tumbling/1.5 h cooking time) and cooked ham with 9 h of tumbling/1.5 h cooking time indicates that the effect of tumbling time was significant on physical characteristics, namely colour and texture. *L** and *b**, Hardness, Gumminess, Chewiness increased with the increase of tumbling time and *a** decreased. The chemical characteristics were less affected by tumbling time since proteolysis of myofibrillar proteins, FAA content and volatile profile were similar in cooked ham that suffered 9 h and 18 h of tumbling. In the cooked hams mentioned above the effect of shelf life was less prominent on physical characteristics, only hardness decreased at 90 days. Most relevant effects of shelf life were observed on chemical characteristics, the proteolysis of myofibrillar proteins and the relative % of some volatiles (mainly, aldehydes) increased at 90 days, whereas FAA decreased (except His that increased).

Organoleptic analyses of mouth texture, colour, flavour and global appreciation highlighted that the physical modifications due to the effect of tumbling time and the chemical modifications due to the effect of shelf life had no significant impact on sensorial characteristics of cooked ham.

The addition of spent yeast extract as an ingredient of cooked ham produced with 9 h of tumbling increased *L**, hardness, gumminess, chewiness, ash and protein contents when compared with control cooked ham with same tumbling and cooking times. Some FAA, namely, Ala, Gly, Val, Ser, Thr, Lys, Trp also increased in cooked ham added with spent yeast extract, whereas qualitative volatile profile was similar. The influence of spent yeast extract addition was prominent when compared with the influence of cooking time. No advantages were observed on increasing cooking higher than 1.5 h (the minimum period to

obtain a microbiologically safe ham product). In general, during shelf life cooked hams with spent yeast extract presented similar evolution as control cooked hams. No significant differences were observed on physical parameters when each cooked ham and respective cooking time was compared at 12 and 90 days, although chemical modifications were observed with respect to increase of proteolysis, His, aldehydes and esters.

For the first time the proteolysis of cooked ham was described by high resolution chromatography assays explaining the influence of tumbling time, yeast extract addition and shelf life on sarcoplasmic and myofibrillar proteins. The addition of spent yeast extract increased the small peptides (<14 kDa PSDB protein fraction) in comparison with typical protein profile observed in conventional cooked ham.

The higher hardness of cooked ham added with yeast extract when compared with control cooked ham is explained by the higher protein content and denaturation due to heat action that strength the gel formed during cooking and improves the textural characteristics of cooked ham. Finally, according with this study, spent yeast extract could be used as gel stabilizer in cooked ham formulations.

Sensorial analyses performed on three types of ham cooked during 1.5 h: *i)* with addition of 1% spent yeast extract and 9 h tumbling time, *ii)* with addition of 1% acetate buffer and 9 h tumbling time and *iii)* conventional ham with 18 h tumbling time performed at 12 days showed that no significant differences were observed between the three types of ham concerning texture and flavour. However, significant differences were observed between cooked ham with and without spent yeast extract with regard to colour and between cooked ham with spent yeast extract and conventional cooked ham in global appreciation being the first one the lower scored by panellists. Although, the three types of ham had a global appreciation higher than 3, which is the minimum satisfactory level. At the end of shelf life the sensorial characteristics of cooked ham added with 1% spent yeast extract were similar to those of cooked ham without spent yeast extract and with those of conventional ham for all attributes and presented scores around 4, that means “Good” in the used scale.

At 12 days the addition of spent yeast extract to cooked ham tumbled during 9 h increased cooked ham hardness, gumminess, chewiness, FAA, <14 kDa PSDB protein fraction, and volatiles when compared with the same cooked ham without yeast extract addition and gave characteristics similar to the conventional cooked ham tumbled during 18 h. At 90

days volatiles and proteolysis of myofibrillar protein fraction increased and FAA decreased and the differences between the three types of ham were attenuated.

Spent yeast extract can be used as an ingredient in the production of cooked ham to reduce the tumbling time of conventional cooked ham. Spent yeast extract does not compromise the physical and chemical characteristics of ham evaluated at the beginning and the end of shelf life and sensorial appreciation is not strongly changed.

12.1. Further works

The further use of spent yeast extracts as an ingredient in cooked ham industry requires some studies that should be performed in the future, namely:

- Trying different compositions of the buffer that is used to perform yeast extraction.
- Characterization of stability of spent yeast extracts and evaluation of its shelf life to be used as a commercial ingredient.
- Evaluate the impact of addition of spent yeast extract in the improvement of cooked ham nutritional value, because *Saccharomyces* is known as a source of important nutrients. Thus, it is expected that yeast extract contain several nutrients, namely, vitamin B, chromium, nucleotides, among others.
- Evaluate the effect of different concentrations of yeast extract on characteristics of cooked ham with different tumbling times.

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